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ON THE RELATION OF POTASSIUM TO THE NEUROLOGICAL MANIFESTATIONS OF HYPOCALCEMIC TETANY*

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Received for publication September 8, 1948

There are many factors recognized to be involved in the production of the neurological manifestations of tetany among which hypocalcemia is generally considered to be the most important. However, under certain circumstances tetany may appear with only slight decreases in the total serum calcium. In other situations more considerable depressions of serum calcium may be present without tetany. Since neuromuscular irritability is influenced by the concentration of ionized calcium, some of these discrepancies are readily explained by the presence of factors which alter the proportions of ionized to unionized or bound calcium in the serum. The serum protein level is of the first importance in this respect. The hydrogen ion concentration of the blood is also significant both in altering the degree of ionization of calcium in the serum and in influencing neuromuscular irritability directly. Alkalosis decreases the proportion of ionized calcium and at the same time directly increases neuromuscular irritability. The development of tetany after vomiting and hyperventilation is due largely to alkalosis. In addition to the absolute level, the rate of change in serum ionized calcium influences the production of symptoms. A sudden reduction in serum calcium from a high level, as after parathyroidectomy for hyperparathyroidism, is more likely to result in tetany than is the same degree of reduction occurring over a considerable period of time.

There are other factors involved in the production of tetany which may not be mediated through changes in the serum calcium. The conversion of latent to overt local or generalized tetany as in the

* A preliminary report was made to the Southern Society for Clinical Research, New Orleans, La., January 27, 1948 and published in Abstract Form, American Journal of Medicine, 4, 455 (1948).

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Trousseau phenomenon is an example. Here the effects of ischemia apparently summate with the low calcium peripherally to convert latent to frank tetany. Epinephrine injected intravenously or intra-arterially similarly will incite tetanic phenomena either generally or locally in the presence of a depressed serum calcium (1). We have seen carpopedal spasm induced in a patient with latent tetany following the subcutaneous injection of histamine and of urocholine. Finally emotional factors possibly acting through sympathetic discharges, hyperventilation or hysteria may precipitate or simulate tetany. In none of these situations has a further change in serum calcium been demonstrated to be responsible for the manifestations.

Experimentally and in the absence of changes in the serum calcium, phenomena which may be interpreted as tetany have been produced by magnesium depletion with resulting low serum magnesium (2). One case of pure hypomagnesium tetany has been reported in the pediatric literature (3). In the presence of a low concentration of calcium in the serum, a concomitant decrease in serum magnesium might be expected to precipitate tetany. As yet there have been no studies to demonstrate this point.

It is clear, therefore, that there are many other factors in addition to the level of the serum calcium which are concerned with the actual precipitation of the neurological phenomena of tetany. The purpose of this report is to describe one more factor which enters into the problem of tetany and which has not received attention in the clinical literature, namely the level of the serum potassium.

The physiological antagonism of calcium and potassium ions in neuromuscular action has been long recognized. Within certain limits an increase in nerve irritability in vitro can be produced by decreasing the calcium ion concentration or increasing the potassium ion concentration. In vivo, diminution of the serum potassium is associated with muscular weakness or paralysis. Depression of serum calcium is associated with tetany. Elevations in calcium may result in some muscular weakness, while elevation in potassium may be followed by either an increase in irritability or weakness.

An opportunity to study the interrelations of calcium and potassium in tetany was afforded us by the observation of two patients with hypocalcemia who developed the low potassium syndrome. In one

tetany could be induced or abolished almost at will by altering the level of serum potassium without changing serum calcium.

The first of the two patients was a 28 year old, single, dwarfed, white female with a long history of diarrhea of undetermined etiology, but possibly pancreatic in origin. She had roentgenological and blood chemical findings consistent with a diagnosis of osteomalacia, i.e. normal serum calcium, low serum phosphate and elevated alkaline phosphatase. Four months before she was seen by us the patient was subjected to a parathyroid exploration with removal of parathyroid tissue because some observers suspected hyperparathyroidism. Subsequently the serum calcium fell from 9.5 to 7.6 mg. per 100 ml. and her serum phosphate rose from 1.6 to 2.2 mg. per 100 ml. About this time the first evidence of diabetes mellitus developed but the disease was not recognized until 3½ months later when the patient returned in coma with severe acidosis. Her blood sugar was 398 mg. per 100 ml. and CO_2 combining power 14 vol. per cent. She was treated vigorously with insulin, intravenous saline, and glucose in the conventional manner but was brought out of ketosis with difficulty. Twenty-four hours after admission when her diabetes had been fairly well controlled she complained of severe weakness and dyspnea with inability to lift her head or arms from the bed. Deep tendon reflexes were depressed. The low potassium syndrome following treatment of diabetic acidosis and accentuated by chronic diarrhea was suspected and confirmed by the finding of a serum potassium level of 1.4 milli-equivalents per liter. Serum calcium at this time was 7.2 mg. per 100 ml. No evidence of tetany was noted. She was given 3 grams of potassium chloride intravenously as a 1 per cent solution following which the paralysis promptly disappeared but she began to complain of severe cramps in the legs. She was noted to have a positive Chvostek sign. The significance of this finding was not appreciated at this time so that correlative studies of serum calcium and potassium levels were not made, nor were careful notes kept of changes in signs and symptoms referable to tetany. She was treated with oral calcium (7.2 grams calcium lactate and 12 grams dicalcium phosphate daily), 1.8 grams of potassium chloride a day and vitamin D (10,000 units per day). Six days after these symptoms were first noted the serum potassium was 2.1 milli-equivalents per liter and serum calcium 6.0 mg. per 100 ml. The patient complained of pain

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in the legs but had neither unusual weakness nor signs of tetany. The next blood chemical determinations recorded were 6 days later when the serum potassium was 4.0 milli-equivalents per liter, and the serum calcium 6.5 mg. per 100 ml. Severe cramping pains in the legs were present and the Chvostek sign was positive. Shortly after this all these symptoms subsided, her serum calcium and potassium having risen to normal levels. The real significance of these findings, however, was not appreciated until 3 months later when the same phenomena were noted in a second patient and an opportunity was afforded to study the calcium-potassium antagonism in greater detail with regard to neuromuscular irritability and changes in the electrocardiogram.

This patient was a 32 year old white married female who had had three previous admissions to Duke Hospital with the diagnosis of nontropical sprue. During previous admissions and on visits to the clinic the patient had been noted frequently to have symptoms of tetany requiring intravenous injections of calcium for relief. She was first seen by us on her fourth admission, which was occasioned by a relapse in her disease. The patient was greatly emaciated on admission, weighing only 76 lbs. She complained of weakness and on the morning after admission it was noticed that she was unable to lift her arms to feed herself. Hypokaliemia was suspected because of these symptoms in a patient with protracted diarrhea and was confirmed by finding a serum potassium of 1.92 milli-equivalents per liter. The serum calcium was 5.7 mg. per 100 ml., total protein 4.6 grams per 100 ml. with 2.1 grams of albumin and 2.5 grams of globulin. There were no signs of tetany at this time. She was given 300 ml. of 1 per cent potassium chloride intravenously. By the time the infusion had ended she had developed severe carpopedal spasm, which slowly subsided during the next 24 hours. The serum calcium was now 5.7 mg. per 100 ml., the serum potassium 2.18 milliequivalents per liter and there were signs of latent tetany, i.e. fasciculation and a positive Trousseau's sign. During the ensuing three months repeated opportunities were offered to observe this phenomenon and a series of studies were carried out as described below.

METHODS

All chemical determinations were carried out by the clinical laboratory of the Department of Biological Chemistry under the direction of

Dr. H. M. Taylor. Serum potassium and sodium levels were determined with a Barnes Flame Photometer. We are indebted to the American Cyanamid Company for this instrument (4). Ionized calcium was estimated from the total calcium and the total protein levels by the use of the nomogram of MacLean and Hastings (5).

Electrocardiograms were recorded with a Sanborn portable cardiette. Q-T intervals were measured and compared to the maximum normal for the patient's sex, age and cycle length, as determined from the table of Ashman and Hull. (6).

RESULTS

Figure 1 records the fluctuations in the patient's symptoms of neuromuscular irritability as correlated with changes in serum calcium and potassium. Concomitant levels of other significant blood constituents are also noted. The solid blocks represent rough estimates of manifestations of either muscular weakness or increased irritability graded from 1+ to 4+. The quantitation of weakness was difficult to estimate and arbitrary. Tetany was scored as 1+ when latent, 2+ fasciculations, 3+ mild carpopedal spasm, and 4+ severe carpopedal spasm. On examining the chart, it will be noted that, by and large, fluctuations in neuromuscular symptoms occurred in relation to the serum potassium levels rather than to the ionized calcium. The latter actually changed very little during these observations. Changes in total calcium paralleled those in total protein, both reaching their highest levels following a period of therapy with concentrated serum albumin between the 23rd and 33rd days. It is realized, of course, that the estimation of ionized calcium by the nomogram of MacLean and Hastings is only a rough approximation under these circumstances, particularly when simultaneous changes in acid-base balance occur. The serum calcium and potassium levels were lowest on admission each time, but due to intravenous potassium chloride administration and the alleviation of the severe diarrhea by therapy, the potassium levels rose more quickly than the calcium. This caused the appearance of the most severe symptoms of tetany during the first few days of each admission. Although the calcium levels rose somewhat with intensive therapy with vitamin D, large amounts of calcium lactate by mouth and frequent intravenous injections of calcium gluconate, they never reached normal levels except immediately after the intravenous medica-

tion. The serum potassium levels were usually moderately to severely depressed, except during or immediately after intravenous potassium injections. During the second admission when the serum calcium levels had become stabilized at a higher level tetany became minimal. The serum potassium, however, had fallen from its highest level. Subsequently both the calcium and potassium rose slightly, but despite this, symptoms of tetany became more severe. It will be noted that at the time the level of ionized calcium had become somewhat more

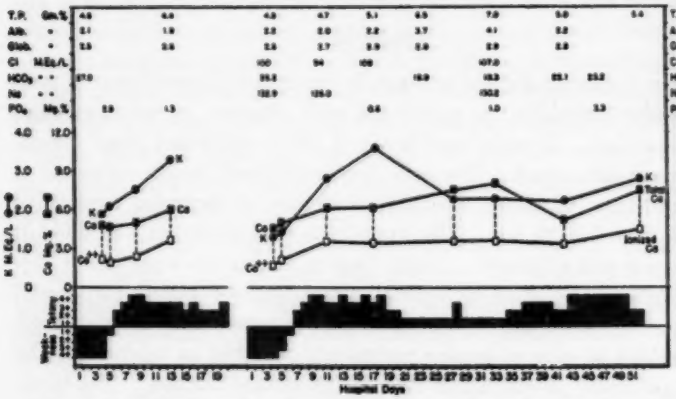

FIG. 1. The relation of the serum potassium and calcium levels to neuromuscular irritability in a case of sprue. See text for description.

stable, the patient was in mild acidosis with a CO_2 combining power value of 18.9 millimols per liter. This would tend to increase ionized calcium, introducing an error into the estimation from the nomogram. Tetany increased in the face of an apparent rise in ionized calcium when the CO_2 combining power had returned to normal, i.e. 25.2 millimols per liter.

Figure 2 records the same serum calcium and potassium data as in the previous figure, correlating them with the changes in the three standard leads of the electrocardiogram. The black bars in the middle part of the figure indicate the maximal duration of Q-T interval for the age, sex and pulse rate of the patient, while the total bar represents the Q-T interval found on each examination. Note that at the beginning

of each admission when the serum potassium and calcium levels were lowest, the electrocardiograms showed the characteristic changes attributed to hypokaliemia. These include depression of S-T segments, low T waves, and prolongation of the Q-T interval (7). With the increase in serum potassium levels, these abnormalities disappeared. Subsequent electrocardiograms, not shown on the chart, have been entirely normal when the serum potassium levels have been 3.5 to 4.0 milli-equivalents per liter.

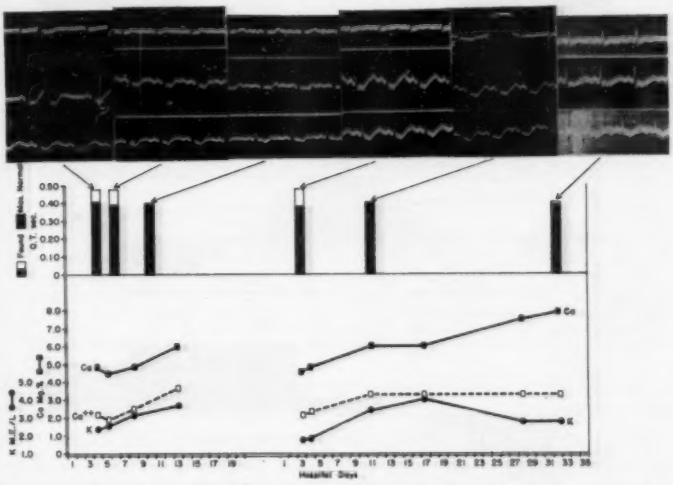

FIG. 2. Electrocardiographic changes and serum potassium and calcium levels in a case of sprue. See text for description.

Figure 3 illustrates the effects of intravenous potassium chloride administration on the signs and symptoms of tetany, the serum calcium and potassium, and the electrocardiograms. Potassium chloride was injected as an 0.5 per cent solution in normal saline over the course of two hours. At the beginning of the infusion the patient exhibited a severe flacid paresis, being unable to lift her head or her arms from the bed. Her serum potassium level was 1.5 milli-equivalents per liter at this time and her serum calcium 4.5 mg. per 100 ml. The electrocardiograms showed evidence of hypokaliemia, i.e. a prolonged Q-T interval, depressed S-T segments and inverted or flat T waves. Within 30

minutes of the beginning of the infusion the paresis had subsided being replaced by increasing signs of tetany. The latter became very severe a little over an hour after the infusion was begun and lasted until the conclusion of the infusion, following which they subsided to some degree. Note that the serum calcium remained fixed while the serum

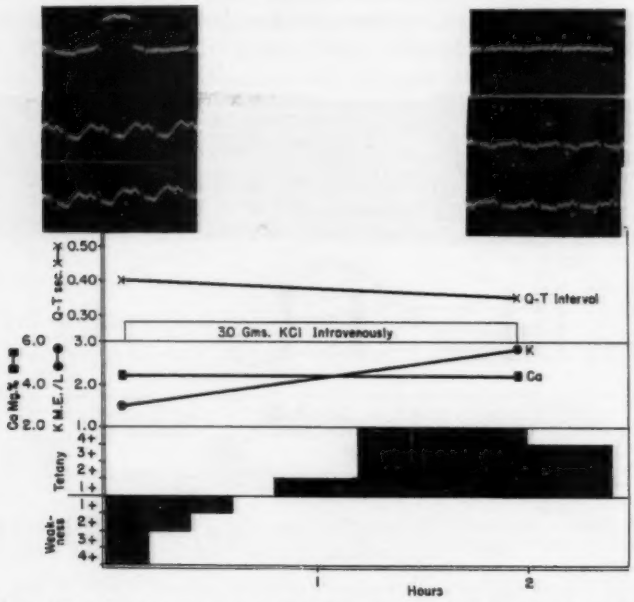

FIG. 3. Effects of intravenous administration of potassium chloride on neuromuscular irritability, serum potassium and calcium levels and the electrocardiogram when patient's symptoms were predominantly those of hypokaliemia. Note development of tetany with no change in serum calcium.

potassium increased from 1.5 to 2.9 milli-equivalents per liter. The Q-T interval of the electrocardiogram returned toward normal. This experiment was repeated 3 times with identical results except that on one occasion the calcium level decreased from 5.3 to 4.8 mg. per 100 ml. during the infusion (Fig. 4). The serum potassium level usually had returned to or near the initial level when next determined, 12 to 24 hours later. On one test the intravenous infusion of 3 grams of potas-

sium chloride in 1500 ml. of saline over 5 hours did not abolish the symptoms of hypokaliemia or elevate the serum potassium. Prior to the infusion the serum potassium was 1.28 milli-equivalents per liter and at the end 1.54 milli-equivalents per liter, an insignificant change. There was no change in serum calcium. On another occasion when there was latent tetany (day 33 of 2nd admission—Fig. 1), slow infusion of 3 grams of potassium chloride in 1300 ml. of saline also had no effect

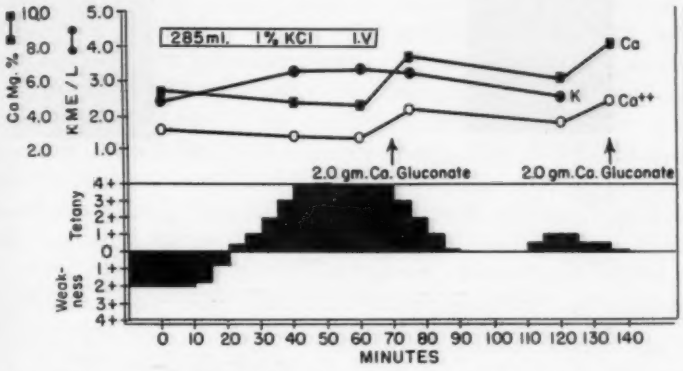

FIG. 4. Production of tetany by intravenous administration of potassium when patient was suffering from hypokaliemia with muscle paresis. Abolition of tetany by calcium gluconate injection intravenously. Note that tetany developed with no appreciable change in calcium and that tetany began to subside after calcium administration before a significant decrease in the serum potassium occurred.

either on symptoms or electrolyte levels. Symptoms and signs of tetany occurred after intravenous potassium infusion only when there was a rise in serum potassium and either no change or a fall in serum calcium.

Figure 4 illustrates the production of tetany by infusion of 285 ml. of 1 per cent potassium chloride in one hour and its alleviation by calcium gluconate before the serum potassium level had changed appreciably. In this experiment the serum calcium level decreased slightly so that it is not possible to attribute the tetany solely to changes in serum potassium.

Figure 5 depicts the effects of 6.0 grams calcium gluconate adminis-

tered intravenously on the signs and symptoms of tetany, the electrocardiogram, and the serum electrolyte concentrations at a time when the patient had severe spontaneous tetany. Tetany was abolished with the rise in serum calcium but there was no change in the Q-T interval of the electrocardiogram or in the serum potassium level.

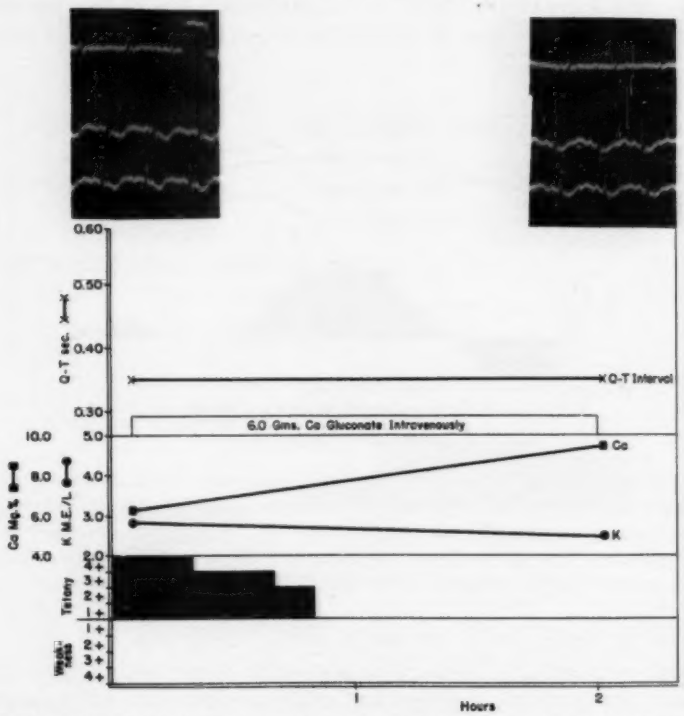

FIG. 5. Effects of intravenous calcium gluconate infusion on the signs of tetany, serum calcium of potassium levels at a time when tetany predominated.

On three occasions 3 grams of potassium chloride and 6–8 grams of calcium gluconate were administered simultaneously by slow drip. In all cases the calcium effect predominated, signs and symptoms of tetany decreasing but never completely disappearing, in contrast to the effect of calcium alone. Calcium levels rose considerably each time.

Serum potassium was unchanged in two experiments while in the third the determinations unfortunately were unsatisfactory.

Two unsuccessful attempts were made to convert local latent to overt tetany by injection of potassium chloride into the brachial artery at a time when the patient exhibited a positive Trousseau's sign. 20 ml. of a 0.01 per cent potassium chloride solution in 0.8 per cent NaCl were injected slowly. Marked flushing of the hand occurred. On the first trial the serum calcium was 7.9 mg. per 100 ml. with a total protein of 7.0 grams per 100 ml. and an arterial potassium level of 2.32 milli-equivalents per liter before injection and 2.56 milli-equivalents per liter in the brachial vein on the same side after injection. On second trial the serum calcium was 7.2 mg. per 100 ml. with a total protein of 5.4 grams per 100 ml., while the arterial potassium was 2.82 milli-equivalents per liter before and 3.20 milli-equivalents per liter afterwards in the venous serum. Since in both experiments the serum calcium levels were somewhat higher than usual we do not consider these results decisive. Subsequently the patient has had a remission in her disease and her electrolyte values have returned to normal and no further opportunity has been presented us to study these phenomena.

DISCUSSION

Alternations in a serum potassium as a factor in tetany have received little attention in the clinical literature. To our knowledge, the reports of Harrison, Harrison Tompsett, and Barr (8, 9) on serum potassium in sprue contain the only mention of this relationship. These workers commented in passing that when their patients were suffering from hypokaliemia they showed none of the manifestations of tetany even though their serum calcium levels were low. They made no observations on the effects of potassium administration. Since our studies were completed two interesting reports have appeared in the veterinary literature describing the induction of tetany in hypocalcemic cattle by the administration of potassium (10, 11). In the syndrome of "grass staggers" due to grazing wheat, Harbaugh and Dennis noted that the serum calcium ranged from 3.3 to 6.6 mg. per 100 ml. in 5 cattle and the serum potassium from 9.6 to 26.6 mg. per 100 ml., most of the potassium values being below normal. The animal with the highest potassium level showed spontaneous tetany and had a potassium-cal-

cium ratio of 4.03 (Normal 1.53). Tetany was induced in the asymptomatic animals by administration of potassium chloride by stomach tube until the K/Ca ratio was about 3.35. When the calcium was reduced by giving oxalates, tetany developed with a ratio as low as 2.62 and a serum potassium level of 9.5 mg. per 100 ml. These findings in cattle are very similar to those in our patient with sprue except that we could not establish a clear relationship between the serum potassium-calcium ratio and occurrence of tetany.

Beyond the potassium ion concentration being a factor in nerve-muscle irritability, our observations do not clarify the exact role of potassium in the body economy with respect to tetany. These data do not permit us to draw any conclusions as to the relative importance of the extra or intracellular potassium ion concentrations in the phenomena described. The fact that tetany was observed after the administration of potassium only when there was a rise in serum potassium does not prove the latter to be the significant change. The serum potassium may begin to rise only after the depleted cellular depots have been replenished to a sufficient degree. The failure to alter the serum potassium significantly on several occasions even after the administration of 3 grams of potassium chloride would support this interpretation. It should be noted that the levels of serum potassium were always low in this study and that the concentrations of potassium associated with tetany were still well below normal. We have no evidence that elevation of serum potassium above normal will induce tetany if serum calcium levels are not reduced. However, there is a suggestion of this in the experiments of Harbaugh and Dennis on cattle (10, 11). These workers reported neuromuscular reactions they interpreted as tetany after administration of potassium chloride without a reduction in serum calcium. While muscle twitching has been noted in some patients with elevated potassium levels, to our knowledge typical carpopedal spasm, Trousseau's phenomenon and Chvostek's sign have not been described in these patients in the absence of either low calcium or alkalosis. These observations suggest that from the clinical standpoint the potassium changes are of greater significance in a negative sense, i.e., a low potassium inhibits hypocalcemic tetany rather than that a high potassium is conducive to tetany when the serum calcium is normal or even slightly depressed. However, conclu-

sive data on this point are not available. The possibility that the administration of potassium induced tetany in our patients by any means other than changes in concentrations of potassium seems unlikely but cannot be ruled out with certainty. In one of our experiments intravenous potassium injection resulted in a decrease in serum calcium level from 5.3 to 4.8 mg. per 100 ml., a change of questionable significance but a phenomenon which has been reported before (12). However, it seems unlikely that such severe tetany as was observed would be induced by such a small change in calcium. Furthermore, the change in calcium was noted only once in six experiments. Finally, the spontaneous increase in serum potassium that occurred in the hospital was associated with increasing symptoms of tetany while the calcium either remained constant or increased slightly. No measurements of blood pH or CO_2 combining power were made before and after potassium administration, but there is no evidence to suggest that significant shifts would have occurred. Darrow has reported on the association between alkalosis and hypokaliemia (13).

One may speculate as to whether the changes in serum potassium bear any relation to previously described observations on tetany. The Trousseau phenomenon by which latent hypocalcemic tetany is converted to overt tetany by the application of a pressure cuff to the arm is a case in point. Under conditions of hypoxia potassium is released from cells. A local increase in potassium at the neuromuscular junctions after the application of the cuff might be suggested as responsible for the local spasm. Our attempts to demonstrate such a phenomenon by the intra-arterial injection of potassium were unsatisfactory. The studies of Lewis (14) and of Kugelberg (15) suggest that the Trousseau phenomenon is due to changes in the proximal part of the nerve rather than at the neuromuscular junctions and may be produced by ischemia of the nerve trunk itself without concomitant ischemia of the rest of the arm. The possibility of local changes in potassium and calcium occurring in the involved stretch of nerve, however, cannot be ruled out by any available data. A similar question may be raised with respect to the induction of local tetany by intra-arterial injection of epinephrine (1), again, without an answer. Epinephrine will cause a release of potassium from the muscle locally (12), although its injection intravenously is associated with a decrease in serum potassium levels, possibly

due to changes in the liver. Until it is possible to measure the electrolyte concentration at the site in the neuromuscular system where the tetanic phenomena are initiated one can only speculate that changes in the concentration of potassium as well as calcium or changes in their proportions are essential features of tetany and that the production of tetany by the various known clinical maneuvers may be mediated through such changes.

These observations on the suppression of hypocalcemic tetany by hypokeliemia undoubtedly have bearing on the variability of appearance of tetany in the presence of low serum calcium associated with different clinical conditions. With the increasing frequency of measurement of serum potassium in clinical practice one may anticipate more correlations of this type. In sprue and steatorrhea tetany is a very variable phenomenon, while hypocalcemia is a very frequent feature of the more severe cases. In the infantile diarrheas, particularly in the postacidotic stage (16) both hypocalcemia and hypokaliemia occur. Rapaport comments on the relative infrequency of the peripheral manifestations of tetany in infantile diarrhea despite low calcium values. He attributes this to a generally lower incidence of peripheral tetany as contrasted with central excitability in the presence of hypocalcemia in infants compared to older children. Since most of the data on infantile tetany have been derived from cases of diarrhea, one wonders whether concomitant hypokaliemia may not be a masking factor. It is not possible to make a definite decision about this from Rapaport's data since not enough simultaneous potassium and calcium determinations were reported. Chronic nephritis is another condition associated with hypocalcemia in which changes in serum potassium occur. Since both hyper- and hypokaliemia occur in this condition (7, 17) one may anticipate that both inhibition and excitation of tetany with the same calcium levels may be seen. We have not had the opportunity to study any such cases.

In view of the severe signs and symptoms of tetany noted in one of our patients following potassium administration, a word of caution is necessary concerning too enthusiastic treatment of the low potassium syndrome under circumstances where there may also be hypocalcemia. When both are present simultaneous administration of calcium and potassium is recommended. In our experience with steatorrhea it is much easier to correct the hypokaliemia than the hypocalcemia.

The abnormalities in the electrocardiograms noted in this study were largely corrected by the administration of potassium, suggesting that their origin was determined by potassium deficiency. The changes were of a type repeatedly described in the literature (7) as associated with hypokaliemia. Certain changes, however, such as a prolonged Q-T interval, have been described under a variety of circumstances other than hypokaliemia, some of which were common to our patient with sprue. Thus a prolonged Q-T interval has been noted with hypocalcemia (18, 19, 20), with acidosis (21, 22), and with severe undernutrition (23, 24), all of which were present to varying degrees in our patient. The prompt shortening of the Q-T interval following the intravenous administration of potassium suggests potassium deficiency as being responsible for this abnormality. There are no data available to demonstrate whether potassium will shorten the prolonged Q-T segment in other conditions in which the serum potassium level is normal. Hypocalcemia did not seem to be a significant factor since the Q-T changes returned to normal both in the acute experiments and during prolonged observations at times when no significant increases in serum calcium had occurred. Furthermore, it has been pointed out recently (25) that the prolongation of the Q-T interval in hypokaliemia is due primarily to a widening of the T wave, whereas in hypocalcemia the Q-T prolongation occurs because of an increase in the RS-T segment in the presence of a normal T wave. In our case, widening of the T-wave accounted for the prolonged Q-T interval and the return to normal was associated with changes in the T wave along. This may be taken as further evidence that hypokaliemia was the chief determining factor in the electrocardiographic changes described. The acidosis in our patient was very mild and was present at a time when the Q-T interval was normal. The undernutrition was a relatively constant factor during most of these studies.

SUMMARY

1. Two cases are reported in which symptoms and a sign of hypocalcemic tetany were masked by the simultaneous presence of hypokaliemia.

2. With the serum ionized calcium remaining relatively constant, fluctuations in signs and symptoms of tetany were correlated with spontaneous increases or decreases in serum potassium.

3. In the presence of hypokaliemia and hypocalcemia without tetany, severe tetany could be induced at will be elevation of the serum potassium by intravenous infusion of potassium chloride. Tetany so induced could be abolished by administration of calcium salts without changes in serum potassium or disappeared spontaneously when the serum potassium returned to its initial low level after infusion without changes in serum calcium.

4. The electrocardiographic abnormalities noted were characteristic of hypokaliemia and were abolished by the administration of potassium but not by calcium.

5. The significance of the serum potassium level in the relation to neurological phenomenal of tetany are discussed in light of these data.

6. The danger of too vigorous therapy with potassium in the presence of hypocalcemia is emphasized.

BIBLIOGRAPHY

1. Harvey, A. M. and Lilienthal, J. L., Jr. Observations on the Nature of Tetany, Effect of Adrenaline. Bull. Johns Hopkins Hosp., **71:** 163, 1942.
2. Kruse, H. D., Orent, E. R., and McCollum, E. Studies on Magnesium Deficiency in Animals. J. Biol. Chem., **100:** 603, 1933.
3. Miller, J. F. Tetany due to Deficiency in Magnesium. Amer. J. Dis. Children, **67:** 117, 1944.
4. Barnes, R. B., Richardson, D., Berry, J. W. Flame Photometry. A Rapid Analytical Procedure. Ind. and Eng. Chemistry, **17:** 606, 1945.
5. McLean, F. C. and Hastings, A. B. Clinical Estimation and Significance of Calcium Ion Concentrations in the Blood. Amer. J. Med. Sci., **189:** 758, 1935.
6. Ashman, R. and Hull, E. Essentials of Electrocardiography. New York. The Macmillan Co., 1937
7. Brown, M. R., Currens, J. H., and Marchand, J. F. Muscular Paralysis and Electrocardiographic Abnormalities Resulting from Potassium Loss in Chronic Nephritis. J. Amer. Med. Ass., **124:** 545, 1947.
8. Harrison, H. E., Tompsett, R. R., and Barr, D. P. The Serum Potassium in Two Cases of Sprue. Proc. Soc. Exp. Biol. and Med., **54:** 314, 1943.
9. Harrison, H. E., Harrison, H. C., Tompsett, R. R., and Barr, D. P. Potassium Deficiency in a Case of Lymphosarcoma with The Sprue Syndrome. Amer. J. Med., **2:** 131, 1947.
10. Harbaugh, F. G., and Dennis, J. The Blood Potassium and Calcium Levels of Cattle Grazing Wheat. Amer. J. Vet. Res., **8:** 396, 1947.
11. Dennis, J., and Harbaugh, F. G. The Experimental Alteration of Blood Potassium and Calcium in Cattle. Amer. J. Vet. Res., **9:** 20, 1948.

12. Fenn, W. O. The Role of Potassium in Physiological Processes. Physiol. Rev., **20:** 377, 1940.
13. Darrow, D. C. Changes in Muscle Composition in Alkalosis. J. Clin. Invest., **25:** 324, 1946.
14. Lewis, T. Trousseau's Phenomenon in Tetany. Clin. Sc., **4:** 361, 1942.
15. Kugelberg, E. Neurologic Mechanisms for Certain Phenomena in Tetany. Arch. Neurol. and Psych., **56:** 507, 1946.
16. Rapaport, S., Dodd, K., Clark, M., and Syllm, I. Postacidotic State in infantile diarrhea: symptoms and chemical data. Postacidotic hypocalcemia and associated decreases in levels of potassium, phosphate and phosphatase in plasma. Am. J. Dis. Children, **73:** 391, 1947.
17. Finch, C. A., Sawyer, C. G., and Flynn, J. M. Clinical Syndrome of Potassium Intoxication. Am. J. Med., **1:** 337, 1946.
18. White, P. D., and Mudd, S. G. Observations on the Effect of Various Factors on the Duration of the Electrical Systole of the Heart as Indicated by the Q-T Interval of the Electrocardiogram. J. Clin. Invest., **7:** 387, 1929.
19. Barker, P. S., Johnson, F. D., and Wilson, F. N. The Duration of Systole in Hypocalcemia. Am. Heart J., **14:** 82, 1937.
20. Wofford, C. P., and Ernstene, A. C. The Diagnostic Significance of an Increased Q-T Interval in the Electrocardiogram. Cleveland Clin. Quart., **8:** 12, 1941.
21. Barker, P. S., Shrader, E. L., and Ronzoni, E. The Effects of Alkalosis and Acidosis upon the human electrocardiogram. Am. Heart J., **17:** 169, 1939.
22. Martin, H. E., and Wertman, M. Electrolyte Changes and the Electrocardiogram in Diabetic Acidosis. Am. Heart J., **34:** 646, 1947.
23. Ellis, L. B. Electrocardiographic abnormalities in severe malnutrition. Brit. Heart J., **8:** 53, 1946.
24. Simson, E., Henschel, A., and Keys, A. The Electrocardiogram of Man in Semi-starvation and subsequent Rehabilitation. Am. Heart J., **35:** 584, 1948.
25. Ernstene, A. C., and Proudfit, W. L. Differentiation of the Changes in the Q-T Interval in Hypocalcemia and Hypopotassemia. Am. Heart Ass. June 18, 1948.

AN INVESTIGATION INTO THE PRESENCE OF ANTIBODIES AND HYPERSENSITIVITY IN THE ENCEPHALITIS PRODUCED EXPERIMENTALLY BY THE INJECTION OF HOMOLOGOUS BRAIN SUSPENSIONS

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The objects of the experiments to be described were:

1. To produce an encephalitis in guinea pigs and rabbits by the injection of homologous brain plus adjuvant. This had already been done by Freund et al (1) and Morrison (2) respectively, on the above animals.
2. To attempt to correlate the presence of circulating antibodies with the clinical signs and histological findings of encephalitis.
3. To attempt to passage circulating or tissue antibody.

In 1932 Hurst (3) produced paralysis in rabbits following repeated subcutaneous and intramuscular injections of heterologous brain, but he found no microscopic lesions in any of his paralyzed animals.

Rivers et al (4) in 1933 produced paralysis and perivascular demyelinization in two out of eight monkeys; and Rivers and Schwentker (5) in 1935 produced signs of involvement of the CNS in six, and found similar histological lesions in seven out of eight monkeys, both groups of workers using repeated intramuscular injections of heterologous brain.

Later Ferraro and Jervis 1940 (6) by the prolonged subcutaneous injections of heterologous brain were able to produce paralysis in monkeys associated with perivascular demyelinization.

The above investigators used heterologous brain tissue in their experiments. In 1947, using homologous tissues and Freund's adjuvant technique (7) (8), Kabat et al (9) and Morgan (10) demonstrated that paralysis and perivascular demyelinization could be produced by the injection of brain tissue into monkeys; Morrison (2) produced paralysis and perivascular demyelinization in rabbits using cord tissue; and

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Freund et al (1) produced paralysis and vascular lesions, but no demyelinization, in guinea pigs using brain tissue.

The production of complement-fixing antibrain antibodies in rabbits following the injection of heterologous brain has been demonstrated by Brandt et al (11) in 1926 and also by Witesbsky and Steinfeldt (12) in 1928; in 1933 Lewis (13), and in 1934 Schwentker and Rivers (15), also showed the presence of circulating complement-fixing antibrain antibodies in the rabbit after the injection of homologous brain tissue. Kopeloff and Kopeloff (14) in 1944, using homologous brain tissue, produced complement-fixing antibrain antibodies in the monkey.

To the author's knowledge there has been, however, little attempt to correlate the presence of circulating antibody with the appearance of paralysis or histological lesions following the injection of brain tissue by the various methods. To date, the only one that is known is that of Freund et al (1) who mention that they found complement-fixing antibrain antibodies in guinea pigs treated with heterologous brain but they concluded that these antibodies were unrelated to the development of the encephalitis. It is true that the complement-fixing and precipitin antibrain antibodies produced in rabbits by Schwentker and Rivers (15) by the intraperitoneal injection of homologous brain [(a) autolysed, (b) virus treated, and (c) alcoholic extracts with pig sera as a conveyor] were associated with paralysis and various non-demyelinated inflammatory lesions in the CNS, but these authors believed that the histological lesions and the paralysis were not directly related to the antigenicity of the brain emulsions injected. Their reasons for this were that the histological lesions were not present in all the paralyzed animals and that they occurred in some of the non-paralyzed animals. Further, they referred to the work of Hurst (3) who found that paralytic accidents may follow injection of substances other than brain, and they concluded that in their experiments the autolysed and virus treated brain injected may have contained toxic substances.

Kabat et al (9) failed to passage the encephalitis experimentally produced in monkeys by the intravenous injection of two normal monkeys with sera, 35 and 40 ccs. respectively, from affected animals, and similarly Morgan (10) failed to passively transfer the encephalitis agent from affected monkeys by injecting two monkeys each with 20 ccs. of serum, intraperitoneally; and Freund's et al (1) attempts at passage

using sera from affected guinea pigs injected into guinea pigs also failed (details not given). However, none of the above investigators tested the serum used in the passage experiments to determine whether or not it contained demonstrable antibody.

METHODS

Experiments I and III were concerned with guinea pigs, and II, IV and V with albino rabbits. Approximate average weights of guinea pigs and rabbits was 370 and 2100 grammes, respectively.

(a) *Preparation of guinea-pig antigen mixture.*

Experiment I. Under aseptic precautions the cerebrums (5 gms.) of two guinea pigs, killed by exsanguination, were freed from meninges as far as possible, and were homogenized in a Waring Blendor with 50 ccs. of normal saline containing 1% phenol to make a 10% emulsion. Twenty ccs. of this emulsion were slowly added to 20 ccs. of warmed Falba,[1] 1 cc. of each at a time, and mixed to a paste-like consistency in a mortar. To this mixture 20 ccs. of heat killed dried tubercle bacilli in suspension in light liquid paraffin oil (0.1 gm. bacilli in 80 ccs. oil) were added, and the 60 ccs. of final mixture ground in a mortar until the whole was of a thin creamy consistency.

The 60 ccs. of brain antigen and adjuvant was heated to 56° for 45 minutes to prevent autolysis, and was stored in the deep freeze.

Experiment III. The preparation of antigen was essentially the same, but the saline emulsion was prepared without the addition of phenol and the final mixture was used immediately and not heated to 56°. This was to eliminate any possibility of the denaturing effect of heat on protein or the production of any "unspecific" reaction by phenol, (11) (17).

(b) *Preparation of rabbit brain antigen mixture.*

Experiment II. The cerebrum (5 gms.) of a rabbit which had been exsanguinated, was treated as described above in Experiment 1 for the preparation of guinea pig antigen, so as to make 60 ccs. of antigen and adjuvant mixture.

[1] Falba (Pfalz and Bauer Inc., New York). A hydrous wool fat-like absorption base.

Experiment IV. Sixty ccs. of rabbit brain antigen and adjuvant mixture was made as described above in Experiment III for the preparation of guinea pig antigen.

Experiment V. The antigenic mixture was prepared in accordance with the directions given by Morrison (2). Two ccs. of freshly ground spinal cord were emulsified with 4 ccs. of a suspension of formalin killed tubercle bacilli in light liquid paraffin oil.

Preparation of Control Injection Mixture.

An adjuvant control injection mixture was prepared for each experiment by methods as described above under experiments I, II, III, IV and V except that in each case normal saline was substituted for the saline brain suspension of the test injection.

Technique of Injection.

In Experiments I, II, III and IV all animals were given at one time three deep subcutaneous nuchal injections of 1 cc. of test mixture or control. Each test animal, therefore, was injected with brain 0.1 gm., saline 1 cc., Falba 1 cc., paraffin oil 1 cc. and tbc 0.0013gms. Because of factors outside the author's control, the test (antigen) and control mixtures of Experiments I and II were left in the deep freeze 14 days before use.

In Experiment V the rabbits received an injection of 0.1 cc. of the test or control mixture into the pad of each foot, i.e. a total of 0.4 ccs. at one time.

Skin Tests.

0.1 cc. of 10% homologous brain emulsion was injected intradermally. In Experiments I and II the animals were also tested by injection with 0.1 cm. adjuvant control mixture. The sites of inection were observed for the prompt anaphylactic type of reaction, and were examined 24 and 48 hours later.

Autopsy and Histology.

Animals were killed when they showed signs of paralysis or were moribund, or after a period of time considered to be sufficient within the limits of the experiment. They were killed by exsanguination

through cardiac puncture and the blood kept for serology in the deep freeze. At autopsy the brain, cord and viscera and sciatic nerves were taken for histology and preserved in 4% formalin-saline. Paraffin sections were stained with haematoxylin and eosin. In addition frozen sections stained with Sudan III were made on all brains, and where indicated, Weils or Mahon's Myelin stain, Alzheimer-Mann, Holzer, Toluidine blue, Mallory's connective tissue, Van Geison and Dublin's reticulin and axis cylinder stains were carried out.

Serology (In all cases blood for serology was taken at time of death).

Complement Fixation Tests. These were carried out on serum, which had been preserved in the deep freeze, using the Eagle modification of the Wassermann test as performed in the Wassermann Laboratory of the Johns Hopkins Hospital (20, 21). The antigen used was a 1–3% saline suspension of homologous brain and the tests were carried out at first qualitatively and then quantitatively.

Flocculation Test. These were carried out using the Eagle Flocculation Test (22). The antigen was prepared by filtering a 10% saline suspension of homologous brain through filter paper and then centrifuging the filtrate at 2000 rpm for ½ hour, the supernatant fluid being used.

Absorption Test. For both complement fixation and flocculation tests homogolous brain or kidney suspensions were used, both being prepared in a manner similar to that used in preparing the original brain antigen suspension before addition of the adjuvant, except that the kidney was well perfused with saline before removal. For absorption ½ cc. of test serum was mixed with ½ cc. of 1/30 brain or kidney emulsion, placed in a water bath at 56° for 2 hours and then left overnight on the bench at room temperature. The following morning the tubes were centrifuged at 2000 rpm for ½ hour and the supernatant sera pipetted off.

Passage Experiments

(a) *Attempt at passage of serum antibody.*

(1) Serum from affected and control animals, respectively, was injected into the exposed jugular veins of normal control animals. By previous experiment it was found that the size of guinea pigs used could stand up to 5 ccs. thus injected at one time. The animals were injected twice weekly, and a skin test

was carried out 24 hours after each injection. When an attempt at intravenous injection failed, the serum was injected intraperitoneally so that in all cases the animal received 10 ccs. weekly.

(2) In accordance with the technique described by Chase (18) serum from the affected animals and controls was injected intradermally into a normal guinea pig and 0.1 cc. of 10% brain emulsion injected into the site 24 hours later, in order to attempt to elicit a Prausnitz-Küstner reaction.

(b) *Attempt at Passage of Cellular Hypersensitivity.* The methods used were those described by Chase (19) and consisted of:

(1) Sick animals, or animals anticipated to be so, were injected intraperitoneally with 20 ccs. of sterile liquid paraffin and killed about 48 hours later. The peritoneal cavities were washed out with 20 ccs. heparinized Tyrode solution containing $\frac{1}{10}$ volume normal guinea pig serum. The cells were separated from the supernatant fluid after standing, and centrifuged. The sediment was washed in heparinized Tyrode solution, and again spun down, and this procedure was repeated two further times, the final sediment being made up to a 3 ccs. suspension with heparinized Tyrode containing $\frac{1}{10}$ vol. normal guinea pig serum. The 3 ccs. suspension was injected intraperitoneally into a test animal; skin tests were carried 24 hours later.

(2) The spleen and a number of lymph nodes from two exsanguinated animals which had shown paralysis, were ground up together and treated with heparinized Tyrode solution as described for the cellular portion of the peritoneal exudates in (1) above. Five ccs. of the suspension were injected intraperitoneally into a test animal, which was skin tested after 24 hours.

(c) *Attempt to Passage Encephalitic Agent.* Two brains weighing about 5 gms. after thin slices had been removed for histological examination, from affected and exsanguinated animals were ground up in 10 ccs. saline and injected intraperitoneally into two normal guinea pigs which were skin tested 24 hours later

CLINICAL SIGNS OF ENCEPHALITIS

Guinea Pigs (see Table III)

(a) *Experiment I.* Ten out of twelve guinea pigs injected with brain antigen and adjuvant showed signs referable to the CNS. The onset

varied from 14 to 28 days after the injection, and in all cases consisted of weakness of the back and hind legs; in addition three cases showed paralysis of the forelegs, two cases marked ataxia, and two cases showed twitching of limbs. As a rule the onset was fairly sudden and the animal became almost moribund within 24 hours, when it was killed. In two cases (Nos. 8 and 10) each animal was allowed to live 5 days, during which time the symptoms became progressively worse.

Two animals (Nos. 4 and 9) showed no signs. No. 4 was killed on the second day because of a limp (misinterpreted as a paralysis), which was afterwards found to be due to a cervical abscess at the site of inoculation. No. 9 was sacrificed on the 42nd day. None of the 12 controls injected with adjuvant mixture showed any signs of CNS involvement, and all were sacrificed on the 42nd day.

(b) *Experiment III.* Fourteen out of twenty test guinea pigs showed symptoms referable to the CNS; all of these showed weakness of hind legs and back and two showed ataxia. The earliest case showed the onset of signs on the 11th day, and the latest on the 21st day. All animals were killed within 24 hours of the onset of the symptoms. Six out of twenty test animals died without symptoms within a period of 14 to 25 days and though no cause for death was found grossly at autopsy, four of the animals were found to have had encephalitis on microscopic examination. The cause of death of the remaining two was not determined. Ten control animals injected with adjuvant mixture only, showed no signs and eight were sacrificed on the 35th day. Two died on the 7th and 20th day respectively but no cause for these deaths could be found.

Rabbits

(a) *Experiment II.* None of the ten test nor ten control animals showed any signs of CNS involvement, and all were sacrificed at about the 10–11th week.

(b) *Experiment IV.* None of the ten test nor five control animals showed any signs of CNS involvement and all were sacrificed at about the 10th to the 12th week, except test animal No. 45 which died on the third day from pneumonia and empyema.

(c) *Experiment V.* None of the three control nor six test animals showed signs of CNS involvement except test animal No. 46 which

showed some weakness of the hind legs on the 11th day following injection. It was killed 24 hours later. The other animals were sacrificed at the 10th to 12th week.

SKIN TESTS

Guinea Pigs (see Table III). Skin tests were performed on the animals of Experiments I and III, as described under "Methods" on the 14th day following injection. All tests were negative at the site of injection of brain suspension alone. As was expected, however, the same animals showed a delayed inflammatory necrotizing reaction at the site of the injection of the adjuvant mixture which contained dead tubercle bacilli.

Rabbits. In Experiments III, IV and V skin tests with brain suspensions alone were negative. An inflammatory delayed necrotizing reaction occurred at the site of the injection of the adjuvant mixture containing dead tubercle bacilli and many of these reactions became indolent ulcers which healed very slowly.

GROSS AUTOPSY FINDINGS

No gross lesions were found in the CNS of either guinea pigs or rabbits.

In all cases in test and control animals a considerable induration measuring up to 1–2 cm. in diameter was found in the muchal subcutaneous tissues at the site of injection of the brain adjuvant emulsion. Very often these areas were cystic and loculated, and contained a creamy material varying in appearances from that resembling the original injection emulsion to a greenish pus.

HISTOLOGY

Guinea Pigs.

In both experiments I and III the positive findings were the same, consisting of acute to subacute focal, perivascular inflammation of the meninges, brain and stem, cerebellum, cord and nerve roots.

In Experiment I, ten out of the twelve test animals showed these lesions. In Experiment III, seventeen out of twenty; none of the twelve and ten controls, respectively, in either experiment showed any pathological lesion.

Brain. This showed a focal encephalitis, which always appeared to be related to the blood vessels. (Fig. 1) The lesions were situated in both white and grey matter, but the areas most heavily affected were the para-ventricular and subependymal tissues (Fig. 4) and the subpial layers of the cortex in relation to the penetrating vessels (Fig. 12).

In the acute phases there was intramural and perivascular infiltration of the vessel wall, mainly with migrating monocytes and polymorphonuclear cells which wandered out into the interstitial tissues (Figs. 2, 3, 4, 5). In H. & E. sections the superficial appearances of such an acute inflammatory condition affecting the vessel wall suggested that an acute necrotization had taken place (Fig. 2). Closer examination and special stains, however, showed that the wall of the vessel was swollen with an excessive outpouring of inflammatory exudate which had separated the collagen fibres without destroying the continuity of the wall. In no cases was it possible to demonstrate a fibrinoid necrosis by special stains. Eosinophiles were rarely found. Endothelial swelling was marked, and was sometimes associated with thrombosis or blockage of the lumen by leucocytic thrombi (Fig. 3).

Haemorrhages were frequent and appeared as small isolated foci in the interstitial tissues around capillaries, or intramurally and perivascularly into the VR spaces of the somewhat larger vessels (Figs. 3, 4, 5).

In the less acute lesions the infiltrating cells were mainly lymphocytes, monocytes and epithelioid cells, with only a few polymorphonuclear cells occasionally. In some cases the vessels showed a perivascular cuffing or lymphocytes or monocytes (Figs. 7, 10) and in others monocytic and epithelioid cells were grouped around the vessels in masses that had the appearance of a granulomatous nodule, sometimes obscuring the lumen (Fig. 6). Giant cells were never demonstrated. Affected vessels in the deeper parenchymatous tissues had a circumscribed nodular appearance with no apparent reaction in the surrounding tissues.

However, beneath the pia and especially beneath the ependyma there was a heavy increase of cellularity of the surrounding tissues due not only to infiltration of monocytic and lymphocytic cells, but also due to some microglial proliferation. In these latter areas proliferating astrocytes could be occasionally demonstrated.

Fat-laden gitter cells were never demonstrated, and exceedingly

rarely there was an apparent demyelinization in a very narrow zone surrounding an acutely inflamed vessel (Fig. 8). In the centres of inflammatory foci some chromatolysis of ganglion cells was occasionally seen, but generally the nerve cell bodies showed no degeneration.

The tela choridea in all cases was focally thickened by a mononuclear cell infiltration and generally the choroid plexus was also involved (23 out of 27 cases affected) (Fig. 9).

As far as could be ascertained the above lesions were always related to blood vessels, but it was often difficult to determine the type of vessel involved, arterial or venous, because of the profound inflammatory changes. The impression was gained, however, that the venules and capillaries were the main vessels affected, although in some areas all vessels including arterioles were involved. Both acute and subacute lesions were often present in the same brain. The lesions were most prominent in the cerebrum and present to a conspicuous, but less degree, in the brain stem (especially around the fourth ventricle). In one case, the lesions were found in the stem only.

Cerebellum. Perivascular infiltration by mononuclear cells, but no polymorphonuclear cells, was encountered in nine out of twenty-seven cases affected (Fig. 10) but in only one or two was there evidence of slight localized degeneration of Purkinje cells.

Spinal Cord. The lesions were demonstrated in the cord in sixteen out of twenty-seven cases affected. Generally they were much milder than those seen in the brain, and in some cases were found only in the upper part of the cord. The grey matter was seldom involved.

Nerve Roots. A radiculitis, involving the nerve roots arising from the cord (Fig. 11) was demonstrated in eight out of the twenty-seven cases with lesions elsewhere.

Meninges. These showed a focal leptomeningitis which consisted of perivascular infiltrates of mononuclear cells, lymphocytes, plasma cells, monocytes, and sometimes epithelioïd cells, affecting heavily the veins and venules (Figs. 12, 13). This focal cellular infiltration extended over the cortex, the base (where it was most severe passing up into the choroidal fissure), the stem and only occasionally did it extend down over the spinal cord. Polymorphonuclear cells were only rarely present amongst the infiltrating cells of the meninges.

Viscera. No lesions were found in the viscera or sciatic nerves of

any of the test or control animals. At the site of the original nuchal injection a granulomatous scleroma adiposum was found subcutaneously in all animals.

Rabbits.

Rabbits 1 and 3 (experiment II) showed a moderate degree of focal meningo-encephalitis. The meninges were focally infiltrated with lymphocytes and the inflammatory process appeared to be extending down the penetrating vessels to the cortical subpial layer. Within the brain there were many circumscribed areas made up of intramural and perivascular infiltrates of lymphocytes, monocytes and epithelioid cells. There was little migration into the surrounding tissues and no neuroglial cell reaction. No lesions were demonstrated in the nervous systems of the other eight test animals and the controls of Experiment II, and no lesions in any test or control animal of Experiments IV and V, except control 74 (Exp. V) which shows a small area of perivascular inflammation (mononuclear cells) in both the pons and cerebrum respectively. There were no visceral lesions demonstrated in any animal other than Rabbit 45 (Experiment IV) which died from a pneumonia, empyema, and focal hepatic necrosis. *Note.* Rabbits 1 and 3 of Exp. 11 were housed in a separate laboratory from the other rabbits of the Experiment.

SEROLOGY

Guinea Pigs (See Table I and III)

(a) *Experiment I.* Of the ten test sera examined, six showed specific complement fixing antibodies to homologous brain antigen. Non-specific antibody reacting with kidney antigen was found in three out of ten sera tested. Neither specific nor non-specific complement fixing antibodies were demonstrable in the sera of twelve control animals nor in the sera of four untreated guinea pigs. No flocculating antibodies to homologous brain or kidney were demonstrated in test, control or normal sera.

(b) *Experiment III.* Of the thirteen test sera examined, six showed specific antibodies to homologous brain antigen. Two of these also had non-specific antibody to homologous kidney. One case out of the remaining seven tested had non-specific antibody to kidney only, and another was anticomplementary. Eight out of ten sera from the con-

TABLE I

Results of the Quantitative Serological Tests on the Sera of Guinea Pigs in Experiments I and III, Injected with Homologous Brain and Adjuvant

EXPER. NO.	GUINEA PIG SERIAL NO.	DAY OF DEATH	COMPLEMENT FIXATION TEST				FLOCCULATION TEST	
			Against brain antigen	Against kidney antigen	Absorbed by brain, against brain antigen	Absorbed by kidney, against brain antigen	Against brain antigen	Against kidney antigen
I	1	22	1:160	0	0	1:160	0	0
	2	15	Not done		—	—	—	—
	3	28	1:120	1:40	0	1:40	0	0
	4	24	1:80	0	0	1:80	0	0
	5	17	1:40	1:10	0	1:20	0	0
	6	22	1:20	1:10	0	1:10	0	0
	7	17	Not done		—	—	—	—
	8	19	0	0	—	—	0	0
	9	42	0	0	—	—	0	0
	10	20	1:10	0	0	0	0	0
	11	14	1:40	0	0	1:20	0	0
	12	17	1:10	0	0	0	0	0
III	43	22	anti-complement		—	—	0	0
	40	22	1:10	1:10	0	0	0	0
	41	25	Not done		—	—	—	—
	50	20	Not done		—	—	—	—
	66	14	1:20	0	0	1:20	0	0
	64	15	Not done		—	—	—	—
	75	15	Not done		—	—	—	—
	68	12	0	0	—	—	0	0
	70	16	0	0	—	—	0	0
	71	14	1:20	1:10	0	1:10	0	0
	67	16	1:40	1:10	0	1:30	0	0
	39	16	0	0	0	0	0	0
	65	22	Not done		—	—	—	—
	29	16	0	0	—	—	0	0
	36	19	1:20	0	0	1:20	0	0
	32	21	1:30	0	0	1:20	0	0
	38	11	1:40	0	—	1:40	0	0
	33	18	0	0	—	—	0	0
	73	14	Not done		—	—	0	0
	34	20	Not done		—	—	0	0

12 control sera of Experiment I, and 10 of Experiment III contained no demonstrable specific antibrain antibodies. Serum for serology was collected in all cases at the time of death.

trol animals were tested, and six of these contained no demonstrable complement fixing antibodies and two were anticomplementary. None of the sera tested contained demonstrable flocculating antibodies.

Rabbits (See Table II)

Complement fixing antibodies to homologous brain antigen were found in all test, control and normal rabbit sera tested. As these positive results were probably due to natural antibody, the sera were heated to 65° for 30 minutes as described by Kidd and Friedenwald (26). After this procedure no specific antibody to brain antigen could be demonstrated in control and normal sera. The remarks below refer to the results of tests carried out after heating.

(a) *Experiment II.* Ten out of ten test animals showed specific complement fixing antibodies to homologous brain antigen and there was no demonstrable non-specific antibody reacting with homologous kidney antigen. Ten out of the ten controls and four out of four normal animals showed no demonstrable antibody to homologous brain although one control serum (No. 12) contained non-specific antibody to rabbit kidney antigen. Nine out of ten test sera examined contained specific flocculating antibody to homologous brain antigen and nine out of ten controls and four out of four normal sera were negative. The tenth control serum contained non-specific antibodies to rabbit kidney.

(b) *Experiments IV and V.* Seven out of nine test sera tested in Exp. IV showed the presence of specific complement fixing antibody to brain; one was negative and a second anticomplementary. In Exp. V six out of six of the test sera examined contained specific complement fixing antibodies. None of the controls in either experiment (5 and 3 animals respectively) contained specific antibodies, but one case (No. 29) contained non-specific antibody to kidney. In the flocculation experiments similar results were obtained, namely, in Exp. IV seven out of nine and in Exp. V six out of six test sera contained specific antibodies whereas all controls were negative.

PASSAGE EXPERIMENTS

(a) *Attempts at Passage of Serum Antibody* (See Table IV)

(i) In Experiment I, one guinea pig (No. 131) received 20 ccs. during a period of 10 days, of pooled serum. This serum was obtained from

TABLE II

Results of the Quantitative Serological Tests on the Sera of Rabbits in Experiments II, IV and V, Injected with Homologous Brain (or Cord) Antigen and Adjuvant

EXP. NO.	RABBIT SERIAL NO.	COMPLEMENT FIXATION TEST				FLOCCULATION TEST			
		Against brain antigen	Against kidney antigen	Absorbed by brain, against brain antigen	Absorbed by kidney, against brain antigen	Against brain antigen	Against kidney antigen	Absorbed by brain, against brain antigen	Absorbed kidney, against brain antigen
II	1	1:30	0	0	1:30	1:6	0	0	1:6
	3	1:160	0	0	1:160	1:48	0	0	1:48
	5	1:30	0	0	1:30	1:4	0	0	1:4
	7	1:30	0	0	1:30	1:2	0	0	0
	9	1:30	0	0	1:30	1:4	0	0	1:4
	11	1:160	0	0	1:160	1:48	0	0	1:48
	15	1:40	0	0	1:40	1:2	0	0	1:2
	13	1:160	0	0	1:160	1:16	0	0	1:16
	17	1:160	0	0	1:160	1:16	0	0	1:16
	19	1:320	0	0	1:320	1:64	0	0	1:64
IV	36	1:10	0	0	1:10	1:2	0	0	1:2
	37	1:80	0	0	1:80	1:4	0	0	1:4
	38	0	0	0	0	0	0	0	0
	39	1:160	0	0	1:160	1:48	0	0	1:48
	40	1:30	0	0	1:30	1:2	0	0	1:2
	41	1:40	0	0	1:40	1:4	0	0	1:4
	42	1:80	0	0	1:80	1:8	0	0	1:8
	43	Anti-complementary			—	1:4	0	0	1:4
	44	1:80	0	0	1:80	1:4	0	0	1:4
	45	not done		—	—	—	—	—	—
V	46	1:160	0	0	1:160	1:20	0	0	1:20
	47	1:160	0	0	1:160	1:20	0	0	1:20
	48	1:160	0	0	1:160	1:8	0	0	1:8
	84	1:80	0	0	1:80	1:8	0	0	1:8
	21	1:160	0	0	1:80	1:16	0	0	1:16
	49	1:160	0	0	1:160	1:20	0	0	1:20

Ten controls in Experiments II, five in Experiment IV, and three in Experiment V contained natural antibody to brain antigen. This antibody was removed by heating to 65° for 30 minutes and subsequently no specific antibrain antibody was found in any control in any experiment. Serum for serology was collected in all cases at the time of death.

the six animals which had demonstrable complement fixing antibodies in their blood, following injection of brain antigen; five of these animals showed histological lesions of the brain.

TABLE III

Summary of the Effects of the Injection of Homologous Brain and Adjuvant into Guinea Pigs of Experiments I and III

EXPER. NO.	SERIAL NO.	WT. IN GMS.	SIGNS AND DAY OF ONSET	MODE AND DAY OF DEATH	SKIN TEST 14TH DAY	SPECIFIC ANTIBRAIN ANTIBODY		HISTOLOGIC LESIONS OF CNS
						CF.	Floc.	
I	1	380	P 22	K 22	0	+	0	+
	2	400	A & P 14	K 15	0	—	—	+
	3	475	P 28	K 28	0	+	0	+
	4	390	0	K 24	0	+	0	0
	5	420	T & P 17	K 17	0	+	0	+
	6	440	P 22	K 22	0	+	0	+
	7	425	A & P 17	K 17	0	—	—	+
	8	475	P 14	K 19	0	0	0	+
	9	425	0	K 42	0	0	0	0
	10	400	P 15	K 20	0	0	0	+
	11	490	P & T 14	K 14	0	+	0	+
	12	375	P 17	K 17	0	0	0	+
III	43	300	A & P 22	K 22	0	Anti-comp.	0	+
	40	300	P 22	K 22	0	0	0	0
	41	300	0	D 25	0	—	—	+
	50	305	0	D 20	0	—	—	+
	66	325	P 14	K 14	—	+	0	+
	64	275	0	D 15	0	—	—	+
	75	300	0	D 15	0	—	—	0
	68	300	P 12	K 12	—	0	0	+
	70	315	P 16	K 16	0	0	0	+
	71	325	P & T 14	K 14	—	+	0	+
	67	325	P 16	K 16	0	+	0	+
	39	275	P 16	K 16	0	0	0	+
	65	275	P 22	K 22	0	—	—	+
	29	320	A & P 16	K 16	0	0	0	+
	36	320	P 19	K 19	0	+	0	+
	32	320	P 21	K 21	0	+	0	+
	38	280	P 11	K 11	—	+	0	+
	33	310	P 18	K 18	0	0	0	+
	73	325	0	D 14	—	—	—	0
	34	325	0	D 20	0	—	—	+

22 Control animals in both experiments gave no reactions clinically, were negative to skin tests, complement fixation or flocculation tests and were negative histologically. Serum for serology was collected in all cases at time of death.

A—ataxia; T—tremors; D—Died; P—paralysis; K—killed.

In Experiment III, two animals (Nos. 80 and 96) received 50 ccs. and 30 ccs. of pooled serum during periods of 35 and 21 days respectively. This serum was obtained from thirteen animals, six of which contained demonstrable antibody in their blood and twelve of which

TABLE IV

Attempt at Passage of Antibody from Affected Guinea Pigs in Experiments I and III

EXP. NO.	POOLED SERA USED	MODE OF INJECTION	TOTAL AMOUNT OF SERA INJECTED	TOTAL PERIOD OF INJECT. (DAYS)	SERIAL NO. OF NORMAL GUINEA PIG INJECT.	SKIN TESTS 24 HR. AFTER INJECT.	CLIN. SIGNS	MODE OF DEATH & DAY FOL. LAST INJECT.	GROSS LESIONS	HISTOLOGY
			cc.							
I	Test	I.V.	20	10	131	0	0	K 31	0	0
	Test	I.V.	5	4	132	0	—	D 2nd inject.	0	0
	Control	I.V.	5	4	133	0	0	D 4	0	0
	Control	I.V.	20	10	134	0	0	K 31	0	0
III	Test	I.V. & I.P.	50	35	80	0	0	K 56	0	0
	Test	I.V. & I.P.	30	21	96	0	0	D 21	0	0
	Control	I.V. & I.P.	50	35	92	0	0	K 56	0	0
	Control	I.V. & I.P.	15	11	42	0	0	D 12	0	0

I.V. and I.P. = Intravenous and intraperitoneal.
K = killed.
D = died.

showed histological lesions of the brain, following injection of brain antigen. Only six out of the thirteen animals which contributed to the pool, had both circulating antibody and histological lesions at the same time.

None of these three animals showed any reaction clinically by skin test. Autopsy and histology were negative. Controls were all negative.

(ii) Ten sera from test animals of Experiment I, and thirteen sera

from test animals of Experiment III, were tested, along with control sera, by intradermal (Prausnitz-Küstner reaction) injection with no positive result. Six of the sera from the test animals of each experiment contained demonstrable antibody

TABLE V

Attempt at Passage of Cellular Hypertensitivity from Affected Guinea Pigs in Experiment III

SERIAL NO. OF TEST ANIMALS USED	MATERIAL USED	SERIAL NO. OF NORMAL GUINEA PIG INJECT.	SKIN TEST 24 HRS.	CLIN. SIGNS	SPECIFIC ANTIBRAIN ANTIBODY		MODE OF DEATH AND DAY	GROSS LESIONS	HISTOLOGY
					CF	Floc.			
67	Spleen and lymph	582	0	Ill 19th day	Ant. com.	0	K 19	0	0
39	nodes	549	0	0	0	0	K 42	0	0
67	Cerebrum	519	0	0	—	—	D 5	0	0
39		98	0	0	0	0	K 42	0	0
43	Periton.	575	0	0	—	—	D 4	—	—
65	Washings	590	0	P 19	0	0	K 19	0	0
36	After I.P.	466	0	0	0	0	K 35	0	0
32	Paraffin	94	0	0	0	0	K 35	0	0

K = Killed.
D = Died.
P = Paralysis.

All the normal animals were injected intraperitoneally with the cellular tissue from affected animals. Serum for serology was collected in all cases at the time of death.

(b) *Attempt at Passage of Cellular Hypersensitivity* (Table V).

(i) Guinea pigs Nos. 65, 36, 32, 43 were injected intraperitoneally with sterile liquid paraffin.

No. 36 received the paraffin 16 days after the original nuchal injection of brain tissue and showed paralysis three days later and was exsanguinated and killed and the peritoneal washings injected into guinea pig No. 46, as described under "Methods." This animal showed no effect from the injection, gave a negative skin test, negative serology at the time of death and showed no gross or histological lesions.

No. 32 was also given the paraffin injection 16 days following the original injection and showed paralysis five days later and was killed.

The peritoneal washings were injected into guinea pig No. 94 which was sacrificed after 35 days and showed no clinical or skin test reactions, negative serology at the time of death, and negative autopsy and histological findings.

No. 65 received the paraffin intraperitoneally eighteen days after the original injection and four days later became paralyzed and was sacrificed. The peritoneal washings were injected into No. 590 which gave a negative skin reaction after 24 hours. Nineteen days later the animal showed some weakness of the back and right hind leg and paralysis of the left hind leg. It was exsanguinated and killed; serology at the time of death was negative and at autopsy the CNS and viscera were also negative and no cause for the illness was demonstrated histologically.

No. 43 also received the paraffin 18 days after the original injection, and showed paralysis after 4 days when it was killed. The peritoneal washings were injected into guinea pig No. 75 which gave no reaction clinically and none to skin test, after 24 hours. The animal died after 4 days and no cause was found either at autopsy nor in subsequent histological examination.

(ii) Guinea pigs Nos. 67 and 39 were killed on the 16th day following the injection of brain tissue, and emulsions were made from the spleen and lymph nodes. These were injected into guinea pigs 582 and 549. Both of these latter animals gave negative skin tests. No. 582 became ill after 19 days and was killed. No lesions were found at autopsy, grossly or histologically. The serum at the time of death was anticomplementary and the flocculation test was negative. No. 549 remained well for 6 weeks when it was sacrificed. Autopsy and histology were negative and serological tests showed no demonstrable antibodies at the time of death.

DISCUSSION

In the two experiments on guinea pigs the first objective of these experiments was attained, namely, an encephalitis was produced in 27 out of 32 animals injected by a single subcutaneous injection of a saline suspension of homologous brain emulsified with a water-in-oil suspension of tubercle bacilli and an absorption base (Falba). Phenolized

and heated suspensions did not differ appreciably in effect from fresh, untreated tissue suspensions (17). Twenty-four of the thirty-two test animals showed signs of paralysis, and in some cases ataxia and tremors. In the majority of cases the signs occurred within the third week, the earliest being on the 11th day and the latest on the 28th. Such an early onset was also found by Freund et al (1) in their experiments on guinea pigs.

Twenty-three out of the twenty-six cases exhibiting paralysis showed lesions of the brain histologically. The occurrence of paralysis in the absence of demonstrable lesions of the brain such as occurred in No. 40 is not an unknown phenomenon following the injection of brain tissue into the experimental animal, and has been well discussed by Hurst (3). It is, of course, possible that more sections of the brain would reveal lesions in such cases.

In addition, six animals in Experiment III died overnight and were not observed to have had signs referable to the CNS. Four of these animals had brain lesions microscopically. Two animals in Experiment I definitely had no clinical signs and no microscopic lesions in the sections that were examined.

Twenty-seven out of thirty-two test animals showed lesions of the CNS, and the main reaction appeared to be a vascular and perivascular inflammation varying from an acute to a subacute stage (longest duration was 28 days), both stages being present in the same brain, indicating that the process was a continuing one. These lesions seemed to be entirely localized to the CNS for a careful search of other organs revealed no other lesions.

In the H and E sections the acute lesions resembled, superficially, those of periarteritis nodosa, but more careful study showed no necrotization of the vessel walls. The main infiltrating cell was the monocyte, although the polymorphonuclears were present in large numbers. Contrary to the description of similar lesions in monkeys by Morgan (10), eosinophils were rarely found. There was often a heavy outpouring of exudate in the Virchow-Robin space and the latter often contained haemorrhage. Pericapillary haemorrhages in the interstitial tissues were common. Associated with the acute lesions there were sometimes minute thrombi, but little perivascular parenchymatous damage of ganglion cells or demyelinization was noted. Necrosis of

brain tissue was never encountered. Microglial proliferation was not a marked feature.

It is true that an apparent demyelinization was demonstrated here and there in a narrow zone around a vessel acutely affected, but none was demonstrated in the interstitial tissues at a distance from the vessels. Further, the absence of fat-laden gitter cells makes it probable that the apparent demyelinization seen in the special stains was due to the superimposition of an acute inflammatory exudate on the perivascular tissues. It was noted in the sections stained for myelin that the guinea pig brain appears to have less myelinated tissue compared with other animals. This may be one of the reasons why demyelinization was not obvious in these experiments and why Freund et al (1) report the complete absence of demyelinization in their similar series of experiments with guinea pigs. The latter investigators suggest that the lack of demyelinization, which is found in other species affected by an experimental encephalitis (2), (9), (10), may be due to a difference in species.

In the less acute lesions the infiltrates were mononuclear varying from a thin lymphocytic perivascular cuffing to a florid granulomatous looking mass. Such lesions have been described previously in experimental encephalitis induced by the injection of brain tissue (5), (9), (10), (23). Freund et al (1) in their guinea pigs describe lesions essentially similar to these. No giant cells were encountered, and they were absent in the histological findings of others who have used the relatively rapid adjuvant technique (1) (2) (10). Earlier workers who have used the prolonged injection technique (5), (6) found giant cells conspicuous in the brain lesions, as did Morrison (2), using the adjuvant technique in rabbits, in one animal surviving 92 days.

Two animals (Nos. 8 and 10) allowed to live for five days after the onset of paralysis. showed no different histological appearances from others which were killed on the day of onset of neurological signs. Neither showed circulating complement fixing antibody at the time of death.

All meningeal infiltrates were mononuclear, with polymorphonuclears almost conspicuous by their absence. Freund et al (1) found that the choroid plexus and cerebellum were unaffected in their series of cases. This was not the case in this series of experiments for the

choroid plexus, especially at its base, was often heavily infiltrated, and the cerebellum also affected. Involvement of choroid plexus and cerebellum is also reported by others (5) (9) (23).

In the three experiments on rabbits the first objective of this investigation was not attained, namely, the production of an encephalitis.

In Experiment V in which the work of Morrison (2) was repeated using the fresh cord plus adjuvant emulsion, one animal, No. 46, showed weakness of the hind legs on the 11th day, but subsequent examination disclosed no histological lesions in the sections studied. The neurological signs in this case may possibly have been due to the effects of the injected brain tissue as discussed by Hurst (3). However the reported results by Morrison who produced lesions in 8 cases out of 10 injected rabbits, were not obtained in the 6 rabbits treated in the present study.

In Experiment II, two rabbits showed perivascular mononuclear cell infiltrates without neurological signs. These two animals were separated from the rest and it is possible that these animals were infected by the encephalotozoon caniculi or some other infectious agent as suggested by Schwentker and Rivers (15) in regard to rabbits with similar lesions in their experiments. It is of interest to note that McCartney (24) found similar lesions in 55% of alleged normal laboratory rabbits.

Regarding the second objective of this investigation, twelve out of twenty-three test guinea pig sera examined showed specific complement fixing antibrain antibodies at the time of death. The titre was fairly low and seemed to have no exact relationship to the period between the time of the initial infection and the onset of paralysis (Table III).

There also appeared to be no exact correlation between the appearance of paralysis or histological lesions and the presence of circulating complement fixing antibody at the time of death, for in 8 cases which showed clinical and histological signs of encephalitis, no antibodies could be demonstrated. (Two of these were allowed to live 5 days after the onset of paralysis and the other six were sacrificed within 24 hours of the onset.)

One case, No. 46, which was killed by mistake and which had a cervical abscess but no microscopic lesions, possessed a titre of 1:80 against brain antigen.

It could of course be argued that the low titre of antibody or the absence of antibody in some of the cases affected, was due to absorption in the antigen-antibody reaction (if the latter is the mechanism by which the lesions are produced).

Flocculating antibodies were not demonstrated in any of the guinea pig sera.

In the rabbit series, all test animals gave specific complement fixing and flocculating antibrain antibodies using saline-brain-suspension as antigen. It is probable that saline-brain-suspension comes under the category of "Wassermann-type substance" which can evoke complement fixing and flocculating antibody formation easily in the rabbits, but not in other animals (16). Schwentker and Rivers (15) were able to produce them in rabbits by the intraperitoneal injection of aqueous brain suspension. It was of interest that all rabbit sera, test, control and normal, contained "natural" complement fixing antibodies to saline-brain-suspension antigen. Rabbit sera is, for reasons unknown, liable to this non-specific deviation of complement (16) (25), but these natural antibodies can be destroyed by heating to 65° for 30 minutes as described by Kidd and Friendenwald (26).

Several workers (11) (12) (13) have shown that brain cortex contains a lipoid which can act as a haptene, and that this is capable of uniting with protein to form complement fixing antibodies which tend to be organ specific. Further, Schwentker and Rivers (15) showed that the specific antigenicity of homologous brain runs parallel to the myelin content, being absent in foetal and newly-born brains and six times more abundant in white matter than in grey matter.

Despite these findings it is not yet established, either by previous investigators or by the present study, that circulating antibodies produced by the injection of brain tissue are the cause of the histological lesions in the brain that result from the injection.

The third objective of this investigation, the attempt to produce the encephalitis in previously normal animals by passive transfer of serum or tissue or blood cells from affected animals, was negative in the twelve animals subjected to the tests.

In the attempts at passage of cellular hypersensitivity (Table V) one of the six animals (No. 590) became paralyzed on the 19th day following injection. It had given a negative skin test, and negative

serology at the time of death. No lesions were found in the brain histologically and the cause of the paralysis is not evident.

Skin tests with brain tissue on all test animals were negative and none exhibited an immediate or delayed reaction indicative of a hypersensitive state. Freund et al (1) also obtained negative results in guinea pigs injected with homologous brain.

Rivers and Schwentker (5), injecting affected brain tissue from test animals intracerebrally into normal animals, were unable to demonstrate an infective agent responsible for the encephalitis.

The injection of affected cerebrum interperitoneally into two normal animals in the present study was negative (Table V).

Histologically, the main lesion of the encephalitis was a vascular inflammation associated with a mononuclear cell reaction. The vascular changes may well be secondary to a primary perivascular inflammatory focus where antibody meets antigen. However, the lesions suggest in the acute stage, a primary vascular inflammation. Such a concept would postulate special properties of the cerebral vessels for no other vascular lesions were encountered in the viscera of affected animals.

Finally, much speculation has taken place over the alleged similarity of appearance of the experimentally acute lesions and those of acute haemorrhagic leuco-encephalitis (27) and acute disseminated encephalomyelitis. The more chronic lesions have been compared to those in multiple sclerosis and Schilder's disease. It is, however, fruitless to conjecture on the relationship of experimental disease to human encephalitis until further light is thrown on the mechanism of the production of the lesion in the guinea pig described in these experiments.

I wish to acknowledge and thank Drs. Arnold R. Rich, Morgan Berthrong and Isobel Morgan for their stimulating advice in writing this paper, and also to Mr. J. H. Strauss, Miss Hazel Lyons, and Miss B. M. Revel for their technical help.

SUMMARY

A meningo-encephalomyelitis was produced in twenty-seven out of thirty-two guinea pigs injected subcutaneously with a saline suspension of homologous brain in water-in-oil emulsion of dead tubercle bacilli.

Specific antibrain complement fixing antibodies were demonstrated

in twelve out of twenty-three test guinea pigs. Eight cases showing lesions had no demonstrable antibody. The present studies have failed to demonstrate a relationship between the meningo-encephalitis and the presence of antibrain antibodies.

Attempts at passive transfer, using sera or living cells, were essentially negative, as were skin tests for hypersensitivity.

Attempts at producing an encephalitis in rabbits using methods similar to the above were, in the main, unsuccessful, although unexplained lesions were found in two test animals and one control animal.

Specific antibrain complement fixing and flocculating antibodies were produced in rabbits as a result of the injecting of saline-brain-suspension plus adjuvant emulsion.

BIBLIOGRAPHY

(1) Freund, J., Stern, E. R. and Pisani, T. M. (1947) Jour. Immunol. **57,** 179.
(2) Morrison, L. R. (1947) Arch. Neurol. and Psychiat. **58,** 391.
(3) Hurst, E. W. (1932) Jour. Hyg. **32,** 33.
(4) Rivers, T. M., Sprunt, D. H., and Berry, G. P. (1933) Jour. Exp. Med. **58,** 39.
(5) Rivers, T. M. and Schwentker, F. F. (1935) Jour. Exp. Med. **61,** 689.
(6) Ferraro, A. and Jervis, G. A. (1940) Arch. Neurol. and Psychiat. **43,** 195.
(7) Freund, J. and McDermott, K. (1942) Proc. Soc. Exp. Biol. and Med. **49,** 548.
(8) Freund, J. and Bonato, M. V. (1944) Jour. Immunol. **48,** 325.
(9) Kabat, E. A., Wolf, A., and Bezer, A. E. (1947) Jour. Exp. Med. **85,** 117.
(10) Morgan, I. M. (1947) Ibid **85,** 131.
(11) Brandt, R., Guth, H., and Miller, R. (1926) Klin Woch. **5,** 655.
(12) Witesbsky, E. and Steinfeldt, J. (1928) Z. Immunitatsforch, **58,** 271.
(13) Lewis, J. H. (1933) Jour. Immunol. **24,** 193.
(14) Kopeloff, L. M. and Kopeloff, N. (1944) Jour. Immunol. **48,** 297.
(15) Schwentker, F. and Rivers, T. M. (1934) Jour. Exp. Med. **60,** 559.
(16) Weil, A. J. (1941) Bac. Rev. **5,** 293.
(17) Stevenson, L. D. and Alvord, E. C. (1947) Am. Jour. Med. **3,** 615.
(18) Chase, M. W. (1947) Jour. Exp. Med. **86,** 489.
(19) Chase, M. W. (1946) Proc. Exp. Biol. and Med. **59,** 134.
(20) Strauss, J. H. (1948) Personal Communication, Wassermann Laboratory, Johns Hopkins Hospital.
(21) Eagle, H. (1934) Jour. Lab. and Clin. Med. **19,** 621.

(22) STRAUSS, J. H. (1937) Am. Jour. Syph., Gon., and Ven. Dis. **21**, 406.
(23) WOLF, A., KABAT, E. A. AND BEZER, A. E. (1947) Jour. Neuropath. and Exp. Neurology **6**, 333.
(24) MCCARTNEY, J. E. (1924) Jour. Exp. Med. **39**, 151.
(25) EAGLE, H. (1932) Jour. Exp. Med., **55**, 667.
(26) KIDD, J. G. AND FRIEDENWALD, W. F. (1942) Jour. Exp. Med. **76**, 575.
(27) HURST, E. W. (1941) Australian Med. Jour. **2**, 1.

ILLUSTRATIONS

FIG. 1. G.P. No. 39. Paralysis 16th day; killed on 16th day. Low power view of cerebral cortex showing an acute vascular and perivascular inflammatory reaction. The infiltrating cells are monocytes and polymorphonuclear cells. There is little microglial response.

FIG. 2. G.P. No. 39. Paralysis 16th day; killed on the 16th day. Cerebrum showing an acute vascular inflammation with intramural and perivascular infiltration of monocytes and polymorphonuclear cells. The appearances are suggestive of periarteritis nodosa, but though the wall may appear destroyed in the photograph, under the microscope the fibres of the vessel wall are seen to be merely widely separated by exudate and special stains show the continuity of the wall to be intact.

FIG. 3. G.P. No. 11. Paralysis 14th day; killed on 14th day. The pons, showing an acute encephalitis. To the left is a large penetrating vessel, the lumen of which is almost occluded by a leucocytic thrombus. To the right is a large vessel showing a recent haemorrhage into the V.R. space. Interstitial pericapillary haemorrhages are frequent and are represented by aggregates of black dots.

FIG. 4. G.P. No. 11. Paralysis 14th day; killed on the 14th day. This shows an acute subependymitis in the tissues bordering the lateral ventricle. There is a heavy perivascular cuffing with monocytes, lymphocytes and polymorphonuclear cells. Most of the cells infiltrating the interstitial tissues are haematogenous but there is some microglial proliferation. The central vessel is thrombosed. At the left centre (in square) is a vessel showing distension of the V.R. space with blood clot. There are many small interstitial haemorrhages not shown in the photograph.

FIG. 5. G.P. No. 11. High power view of the vessel depicted in Fig. 4 by square. Blood clot is distending the V.R. space.

FIG. 6. G.P. No. 29. Paralysis 16th day; killed on the 16th day. High power view of one of the perivascular granulomatous-like lesions. The infiltrating cells are monocytes and epithelioid cells.

FIG. 7. G.P. No. 11. Paralysis 14th day; killed on the 14th day. Two vessels in the cerebrum showing heavy intramural and perivascular infiltrates of mononuclear cells. The smaller vessel is occluded. A neighbouring ganglion cell has a pyknotic nucleus. Figs. 3, 4, 5 show an acute inflammatory reaction taking place in the same animal.

FIG. 8. G.P. No. 39. Paralysis 16th day; killed on 16th day. Cerebral cortex showing a narrow zone of apparent demyelinization. Such appearances as these were of infrequent occurrence. No gitter cell response could be demonstrated histologically in this animal.

FIG. 9. G.P. No. 7. Paralysis 17th day; killed on the 17th day. Choroid plexus (lateral ventricle) showing a mononuclear infiltration.

FIG. 10. G.P. No. 11. Paralysis on 14th day; killed on 14th day. Shows a perivascular cuffing of a cerebellar vessel.

FIG. 11. G.P. No. 36. Paralysis 19th day; killed on the 19th day. Nerve root from the cervical cord, showing a radiculitis.

FIG. 12. G.P. No. 6. Paralysis 22nd day; killed on 22nd day. Meninges of the choroidal fissure showing a thickening due to a dense mononuclear cell infiltration. Note the penetrating vessel which shows intramural and perivascular mononuclear cell infiltration.

FIG. 13. G.P. No. 10. Paralysis 15th day; killed on 20th day. Shows a mononuclear infiltration of the meninges over the medulla.

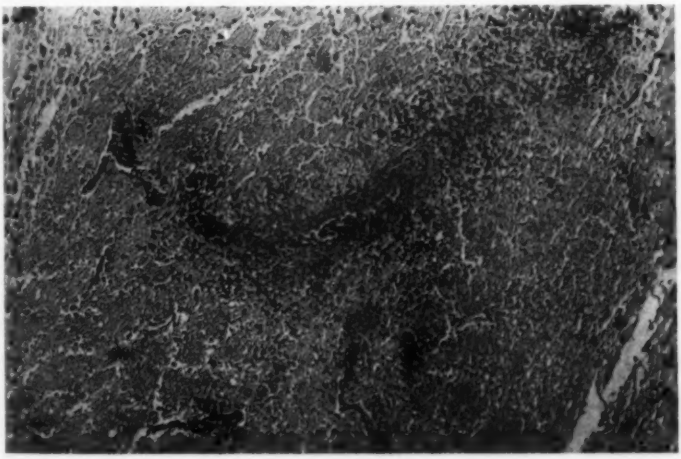

FIG. 1

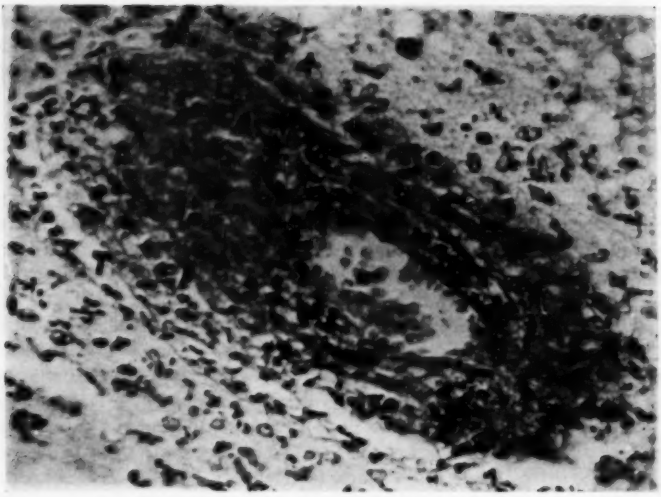

FIG. 2

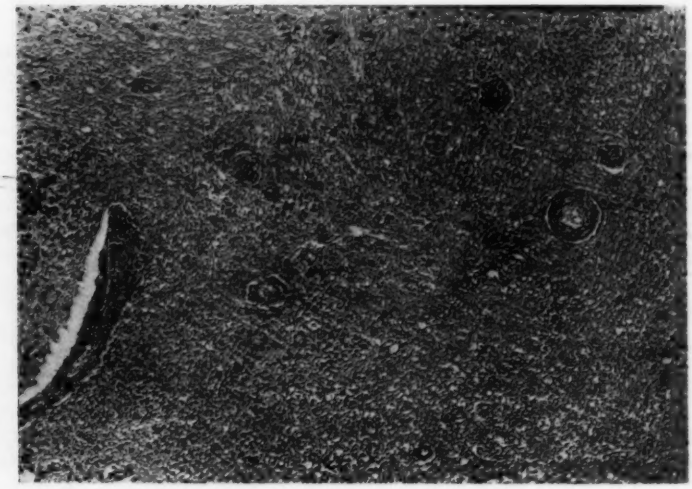

FIG. 3

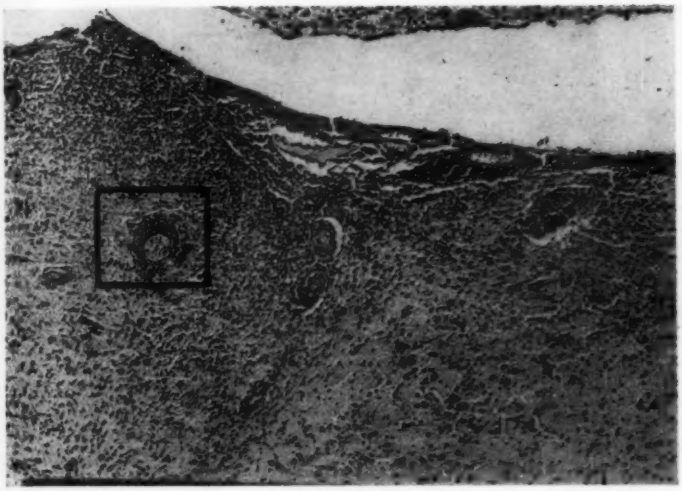

FIG. 4

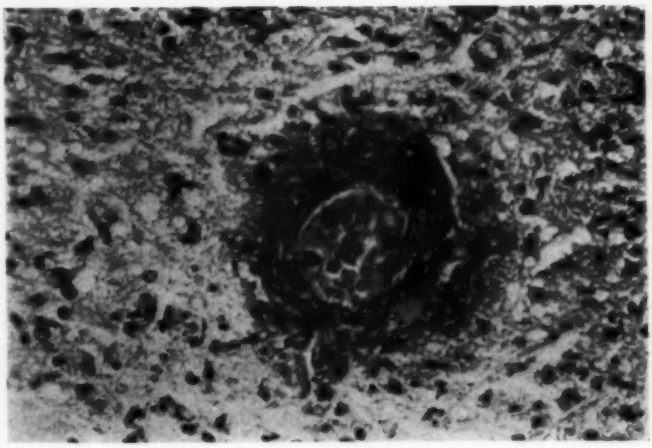

FIG. 5

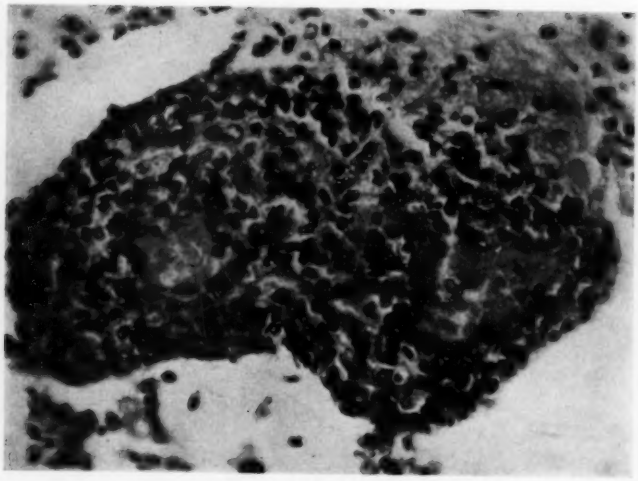

FIG. 6

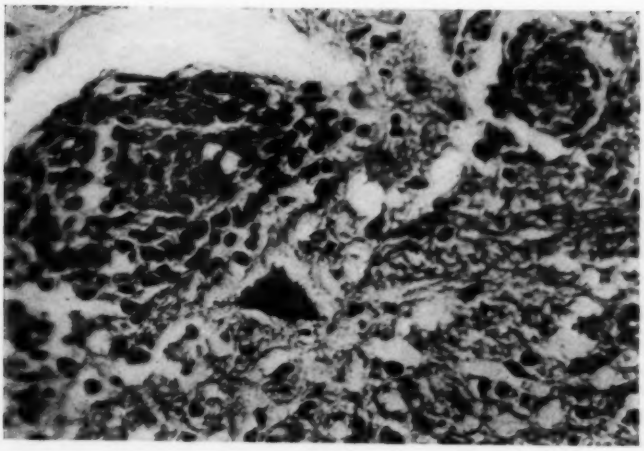

FIG. 7

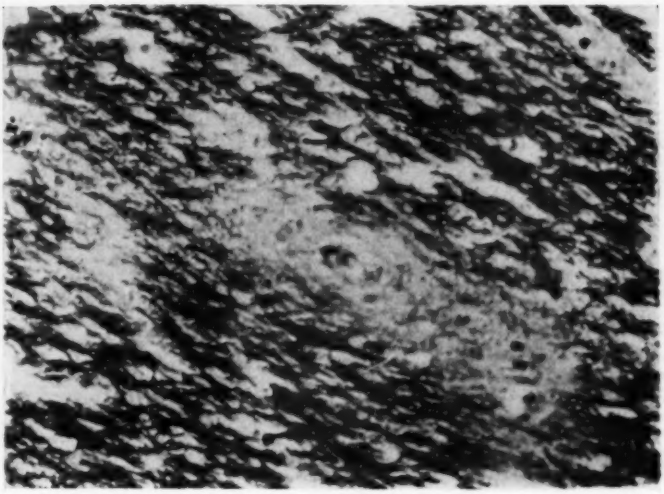

FIG. 8

FIG. 9

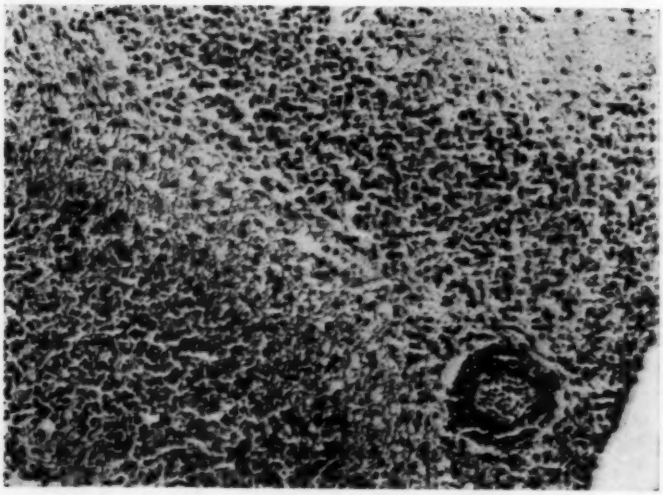

FIG. 10

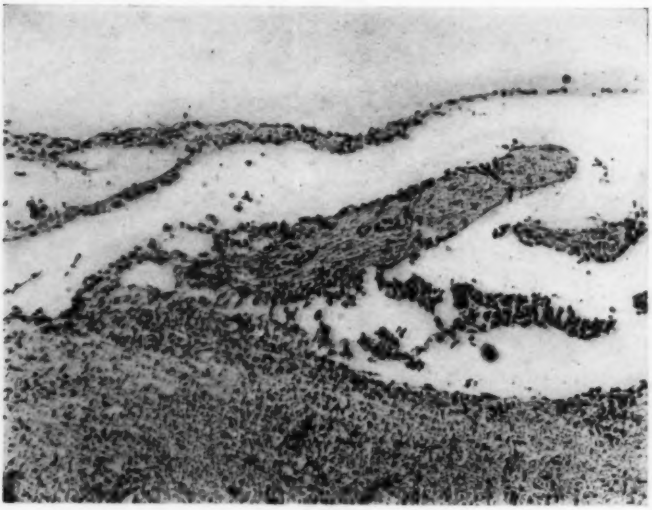

FIG. 11

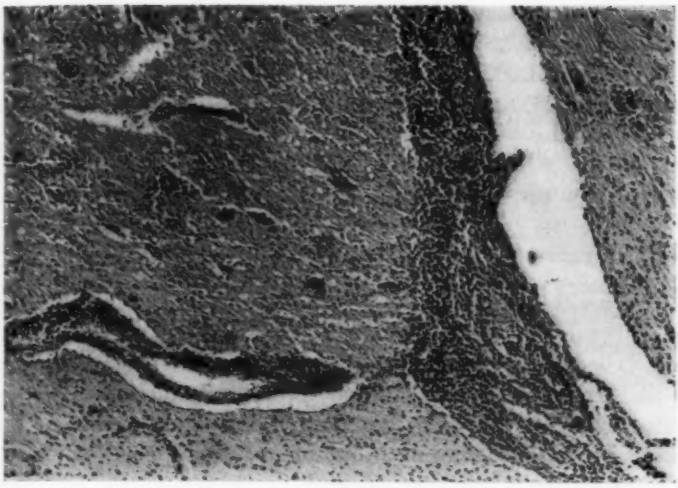

FIG. 12

FIG. 13

ANTI-PNEUMOCOCCAL POWERS OF THE BLOOD IN ALLOXAN DIABETES IN THE RABBIT

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INTRODUCTION

In a previous paper (1) it was pointed out that the method of producing diabetes with alloxan made it possible to investigate, under controlled conditions, the notorious susceptibility of diabetics to infection. In that paper the results of a study of antibody formation were presented, and were: that there is no impairment of antibody formation in rabbits with alloxan diabetes. The present paper gives the results of an investigation of phagocytosis under similar conditions.

Quite soon after the suggestion of Lassar in 1904 (2) that it was the increased sugar content of the blood and tissues that favored bacterial growth, evidence began to accumulate that such an explanation of diabetic susceptibility to infection was too simple. Phagocytosis was studied by Da Costa (3) and Da Costa and Beardsley (4) and they found that the opsonic index in patients with diabetes mellitus was low. In 1911 Handmann (5) found that when he added glucose to normal blood so that a concentration similar to that in diabetic blood was produced, the bactericidal power of the blood for staphylococci was not decreased, nor was the growth of the implanted bacteria favored. Similar results were obtained by Hirsch-Kauffman and Heimann-Trosien (6) when they produced hyperglycemia, lipemia, and acidosis artificially. The latter authors found that hyperglycemic blood from diabetic children without acidosis did not cause an increase in the growth of implanted bacteria when compared with normal human blood, but that blood from three comatose patients did so. After the same patient had been treated with insulin and brought out of coma, bacterial growth in their blood was the same as in normal blood. About the same time the effect of insulin upon phagocytosis was studied

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by Bayer and Form (7, 8) and was found to be stimulating to the phagocytic power of normal horse leycocytes in vitro, and to raise the opsonic index of pancreatectomized dogs.

In 1933 Richardson (9) reported the results of his studies of specimens from human diabetics and from experimental animals, and stated that: "Diabetic whole blood, regardless of the level of its blood sugar, has in general a weaker bactericidal property than has non-diabetic whole blood as tested by standard methods."

In the absence of complete agreement by investigators and because of the lack of clear cut results it seemed to A. R. Rich (10) that: "The matter is of sufficient interest and importance in relation to the problem of resistance to tuberculosis and to other infections to warrant further careful study." At Dr. Rich's suggestion the following experiments were undertaken.

MATERIALS AND METHODS

Male albino rabbits were made diabetic by the intravenous injection of alloxan in 4 per cent solution in saline. In most of the animals a single dose of between 100 mgm per kgm. and 200 mgm per kgm. was used, but in some of the animals which had received the smaller doses a second and sometimes a third dose was required. Occasionally an animal could not be made diabetic. The animals were not fasted before administration of the alloxan, and glucose and insulin were not used. Blood sugar estimations were carried out by the method of Folin and Malmros (11). The specimens were not fasting samples of blood and animals with sugar levels below 300 mgm per cent were not included in the experiment. A certain number of rabbits died of diabetes quite soon after having been injected. The ones used in these experiments, however, had all survived, without treatment and with continuous hyperglycemia, polyuria and glycosuria, for at least five months. None had acidosis. Some maintained their weight, others became thin. Diabetics and normal controls were fed an unlimited diet of Purina Laboratory Chow and water. Polydipsia was marked in the diabetics. Some of the diabetic animals had been used in previous experiments in which they had been immunized against crystalline egg albumen. The level of their serum proteins was normal.

The bactericidal powers of the blood of the diabetics and of normal

TABLE I

	NUMBER OF COLONIES COUNTED IN POURED BLOOD AGAR PLATES INOCULATED WITH 1 C.C. OF DILUTIONS LISTED				W.B.C. PER CU. MM.	BLOOD SUGAR	URINE SUGAR	WEIGHT KG.	DURATION OF DIABETES	INGESTED COCCI PER POLY-MORPH
	— 1/50,000	1/100,000	1/1 million	1/10 million					mos.	
Mar. 6, 10:30 am										
Rabbit 23 D.	134	55	5	—	6,800	433	+++	3.0	5	1.3
Rabbit 57 N.	80	52	4	—	8,400	90	0	4.1	—	3.9
6 pm										
Rabbit 23 D.	too many	too many	600	—						
Rabbit 57 N.	"	"	311	—						
Mar. 16, 11:30 am										
Rabbit 6 D.	120	48	3	0	6,600	390	+++	3.85	12	3.2
Rabbit 55 N.	116	45	4	0	8,000	105	0	3.1	—	3.8
7 pm										
Rabbit 6 D.	too many	too many	272	29						
Rabbit 55 N.	"	500	80	9						
Mar. 22, 11 am										
Rabbit 51 D.	118	77	10	2	6,000	340	++	2.3	6	7.6
Rabbit 57 N.	137	86	10	0	5,940	90	0	4.1	—	4.4
6 pm										
Rabbit 51 D.	too many	too many	496	46						
Rabbit 57 N.	"	"	196	20						
Mar. 23, 11 am										
Rabbit 21 D.	168	76	9	0	9,000	393	+++	3.3	5	8.5
Rabbit 57 N.	170	69	9	0	8,400	90	0	4.1	—	7.9
6 pm										
Rabbit 21 D.	too many	too many	460	62						
Rabbit 57 N.	"	"	205	27						

Rabbit 57 N	170	69	9	0	8,400	90	0	4.1	—	7.9
6 pm										
Rabbit 21 D	too many	too many	460	62						
Rabbit 57 N	"	"	205	27						
Apr. 27, 11:30 am										
Rabbit 27 D	57	30	1	0	7,000	366	+++	2.0	7	1.3
Rabbit 18 N	55	26	2	0	7,2000	100	0	3.2	—	1.5
6 pm										
Rabbit 27 D	too many	650	85	12						
Rabbit 18 N	"	271	33	3						
Apr. 28, 10:50 am										
Rabbit 34 D	87	37	6	1	9,900	363	+++	3.05	7	2.4
Rabbit 54 N	78	31	5	0	6,600	95	0	3.1	—	4.7
6:15 pm										
Rabbit 34 D	too many	too many	944	100						
Rabbit 54 N	"	"	325	38						
Apr. 29, 10:30 am										
Rabbit 9 D	87	31	3	0	9,600	433	+++	3.35	12	3.1
Rabbit 55 N	84	41	5	1	10,400	105	0	3.1	—	3.1
5 pm										
Rabbit 9 D	too many	too many	287	33						
Rabbit 55 N	510	244	32	2						
Apr. 30, 10:15 am										
Rabbit A D	86	35	3	1	7,000	450	+++	2.3	20	2.1
Rabbit 53 N	66	31	4	0	13,000	90	0	2.8	—	1.5
8:45 pm										
Rabbit A D	too many	too many	231	25						
Rabbit 53 N	81	13	3	0						

D—Diabetic; N—Normal.

controls were tested against a type II pneumococcus, of low virulence and with a small capsule. When heavy growth of this organism occurred in a sample of blood, methaemoglobin production caused brownish-black discoloration of the blood, and this was a valuable naked eye indication of the progress of the experiments to be described.

In each experiment, in which aseptic precautions were observed throughout, 5–6 ccs. of blood were collected from an ear vein of a diabetic rabbit in a sterile tube containing 0.1 cc. of heparin (Abbott). A similar sample was collected from a normal control, and total white cell counts were done on each. Then 3 ccs. from each were measured with a pipette and were placed in Wassermann tubes sealed with rubber bungs. Each of these tubes was inoculated with 0.1 cc. of the overnight growth in ascitic fluid broth of the pneumococcus, and the tubes were rotated in the incubator at 37°C for a few minutes to produce mixing. Then a sample of 0.1 cc. was withdrawn from each tube and the tubes were then returned to the 37°C incubator, where they were rotated mechanically at the rate of 8 revolutions per minute. Serial dilutions were then made of the samples that had been withdrawn, using peptone water as the diluent, and blood agar pour plates were inoculated with 1 cc. each of dilutions of $\frac{1}{50,000}$, $\frac{1}{100,000}$, $\frac{1}{1,000,000}$, $\frac{1}{10,000,000}$, of the two samples. The plates were incubated at 37°C for 24 hours and were then stored in the ice box to enhance the zone of hemolysis around the colonies of pneumococci. The colonies were then counted.

The tubes of blood which had been returned to the incubator were rotated until there was an appreciable color difference. As a rule about 7½ hours of incubation were necessary. During the first 1–1½ hours smears were made and were stained by Wright's method. Then the average number of pneumococci taken up by each polymorph was estimated from a count of the bacteria contained in 100 polymorphs. When a color difference was observed another sample of 0.1 cc. of blood was taken from each tube and a second series of dilutions, similar to the first, was made and blood agar pour plates were inoculated with 1 cc. of each dilution. These were then incubated, and the colonies were

TABLE II

Plasma

	NUMBER OF COLONIES COUNTED IN POURED BLOOD AGAR PLATES INOCULATED WITH 1 C.C. OF DILUTIONS LISTED				BLOOD SUGAR	URINE SUGAR	WEIGHT KG.	DURATION OF DIABETES
	1/50,000	1/100,000	1/1 million	1/10 million				mos.
April 2, 11:30 a.m.								
Rabbit 6 D.	120	64	6	0	390	+++	3.85	12
Rabbit 53 N.	147	89	11	0	90	0	2.8	—
7 p.m.								
Rabbit 6 D.	too many	too many	62	7				
Rabbit 53 N.	" "	" "	581	63				
April 9, 11:30 a.m.								
Rabbit 9 D.	61	24	4	0	433	+++	3.35	12
Rabbit 54 N.	51	24	3	0	95	0	3.1	
7 p.m.								
Rabbit 9 D.	too many	too many	386	45				
Rabbit 54 N.	" "	" "	928	137				
April 1, 12 noon								
Rabbit 51 D.	145	68	8	1	340	++	2.3	6
Rabbit 55 N.	169	68	8	0	105	0	3.1	
7:30 p.m.								
Rabbit 51 D.	too many	too many	345	43				
Rabbit 55 N.	" "	" "	321	50				
March 30, 12 noon								
Rabbit 28 D.	161	86	9	2	320	++	3.2	7
Rabbit 57 N.	166	87	15	2	90	0		
7 p.m.								
Rabbit 28 D.	too many	too many	204	27				
Rabbit 57 N.	" "	" "	213	28				
April 13, 10:30 a.m.								
Rabbit 27 D.	103	67	4	1	366	+++	2	7
Rabbit 18 N.	97	45	5	0		0		
6:30 p.m.								
Rabbit 27 D.	too many	too many	197	20				
Rabbit 18 N.	" "	" "	655	78				

TABLE II—*Concluded*

	Number of colonies counted in poured blood agar plates inoculated with 1 c.c. of dilutions listed				Blood sugar	Urine sugar	Weight kg.	Duration of diabetes
	1/50,000	1/100,000	1/1 million	1/10 million				
								mos.
April 19, 11:30 a.m.								
Rabbit 21 D.	77	36	8	1	393	+++	3.3	5
Rabbit 57 N.	133	64	9	1	90	0	4.1	
7 p.m.								
Rabbit 21 D.	too many	too many	226	34				
Rabbit 57 N.	" "	" "	384	62				
April 20, 11:15 a.m.								
Rabbit 34 D.	75	32	7	0	363	+++	3.05	7
Rabbit 54 N.	61	22	2	0	95	0	3.1	
6:30 p.m.								
Rabbit 34 D.	too many	too many	409	47				
Rabbit 54 N.	" "	" "	303	35				
April 21, 11 a.m.								
Rabbit A D.	109	42	0	1	450	+++	2.3	20
Rabbit 53 N.	87	48	5	0	90	0	2.8	
6 p.m.								
Rabbit A D.	too many	too many	329	45				
Rabbit 53 N.	" "	" "	201	19				

D—Diabetic; N—Normal.

counted after storage in the cold exactly as was done in the case of the plates made from the samples taken before incubation.

RESULTS

The results of eight experiments, with relevant data about the animals, are summarized in Table I. In the case of the diabetic animals, the blood sugar figure quoted is an average of several estimations during the period of the experiments. In the case of the normal animals only one blood sugar estimation was carried out. On plates from the lower dilutions after incubation there were so many colonies that they

were uncountable. It will be seen from the table that in every case the growth in the diabetic blood after incubation was two or three times as heavy as that in the normal, although the initial bacterial counts were approximately equal.

To test whether the above findings with whole blood were related to the sugar content of the blood, eight experiments were carried out in the same way, except that plasma was used instead of whole blood for, because of the great diffusibility of glucose, the sugar content of the plasma is approximately the same as that of whole blood. Table II, however, shows that, in the absence of cells, quite different results were obtained. In the plasma experiments the amount of bacterial growth in the diabetic and control specimens was approximately equal, or a heavier growth was obtained in the normal plasma.

DISCUSSION

In interpreting our results we have attempted to bear in mind that alloxan diabetes may differ in some ways from human diabetes mellitus, and that the metabolism of the rabbit differs in certain respects from human metabolism. Also that, though only some of our animals were emaciated, non-specific effects upon the defense mechanisms as result of malnutrition, such as have been described by Gellhorn and Dunn (12, 13), and Berry, Davis and Spies (14), may have had some influence. All our experiments were made with pneumococci, and the phagocytes have been found to be essential for the defense of the body against pneumococci. Rich and McKee (21) have shown that animals deprived of leucocytes will succumb to infection with even completely avirulent pneumococci. The same investigators (22) have also shown that, even in the highly immunized body, removal of the phagocytes while leaving the antibody intact destroys the body's resistance to virulent pneumococci. Wood and his colleagues (15, 16, 17, 18) have shown that while, as is well known, phagocytosis of encapsulated pneumococci does not usually take place outside the body in the absence of opsonin, in vivo it can occur in the complete absence of antibody if the pneumococci can be cornered in the alveoli by the leucocytes. The great importance of leucocytic action in the defense against pneumococci is thus obvious. In earlier experiments (1) we could demonstrate no defect in antibody production in alloxan diabetes, and in the experi-

ments which we have just described we were fortunate in having a pneumococcus with a small capsule, which was susceptible to phagocytosis in vitro in the absence of antibody. The difference between the growth of this organism in diabetic whole blood and in diabetic plasma points to some alteration in the activity of the blood cells in alloxan diabetes. It is unlikely that an alteration in the red cells is responsible for the effect, and we think that some alteration in the function of the white cells must be responsible for these results. The smears which we made during incubation did not demonstrate any complete, or even marked, loss of phagocytic power in the diabetic leucocytes; thus it seems that the defect may be an inability to destroy ingested bacteria rather than a loss of phagocytic power. At present we are quite uncertain whether this abnormality of leucocytic function is associated with excess of glycogen in the blood leucocytes, such as has been reported by Neukirch (19) in human diabetes mellitus, for we had great difficulty in staining glycogen in blood smears; and in sections of the buffy coat, stained by the Bauer method using periodic acid and leucofuchsin (20), we found no convincing difference between the normal and the diabetic leucocytes. Further studies are to be undertaken to investigate the leucocytic abnormality. The in vitro metabolism of the diabetic's leucocytes will obviously be a matter of particular interest.

SUMMARY

An encapsulated type II pneumococcus, of low virulence, when inoculated into the whole blood of rabbits with alloxan diabetes and incubated in vitro, grew much more rapidly than it did when incubated under similar conditions in normal rabbit blood. When these experiments were repeated using plasma instead of whole blood, growth tended to be heavier in the normal plasma. The lowered bactericidal power of the whole blood of the diabetic animals did not appear to be due to a decrease in phagocytic activity. These results are interpreted as indicating a defect in the leucocytic defense of animals with alloxan diabetes. The precise nature of the defect requires further study.

REFERENCES

1. PAYNE, T. P. D. AND CRUICKSHANK, A. H. Bull. Johns Hopkins Hosp. In press.

2. Lassar, O. Dermat. Ztschr. **11:** 189, 1904.
3. Da Costa, J. C. Am. J. Med. Sci. **134:** 57, 1907.
4. Da Costa, J. C. and Beardsley, E. J. G. Am. J. Med. Sci. **136:** 361, 1908.
5. Handmann, E. Deut. Arch. f. klin. Med. **102:** 1, 1911.
6. Hirsch-Kauffman, H., and Heimann-Trosien, A. Klin. Wchnschr. **5:** 1922, 1926.
7. Bayer, G. and Form, O. Deut. Med. Wchnschr. **52:** 784, 1926.
8. Bayer, G. and Form, O. Ibid. **52:** 1338, 1926.
9. Richardson, R., J. Clin. Invest. **12:** 1143, 1933.
10. Rich, A. R. The Pathogenesis of Tuberculosis, 1st Edition, Charles C. Thomas, Springfield, Ill., 1946.
11. Folin, O. and Malmros, H. J. Biol. Chem. **83:** 115, 1929.
12. Gellhorn, E. and Dunn, J. O. J. Nutrition. **13:** 317, 1937.
13. Gellhorn, E. and Dunn, J. O. Ibid. **14:** 145, 1937.
14. Berry, L. J., Davis, J., and Spies, T. C. J. Lab. Clin. Med. **30:** 684, 1945.
15. Wood, W. B. J. Exp. Med. **73:** 201, 1941.
16. Wood, W. B. and Irons, E. N. Ibid. **84:** 365, 1946.
17. Wood, W. B., McLeod, Charlotte, and Irons, E. N. Ibid. **84:** 377, 1946
18. Wood, W. B., Smith, M. R., and Watson, B. Ibid. **84:** 387, 1946.
19. Neukirch, P. Ztschr. f. klin. Med. **70:** 251, 1910.
20. Lillie, R. D. Histopathologic Technic. The Blakiston Company, Philadelphia, 1948.
21. Rich, A. R. and McKee, C. M. Bull. Johns Hopkins Hosp., 1939, **64:** 434.
22. Rich, A. R. and McKee, C. M. Bull. Johns Hopkins Hosp., 1934, **54:** 277.

THE EFFECT OF EXERCISE ON THE RENAL MECHANISM OF ELECTROLYTE EXCRETION IN NORMAL SUBJECTS[1]

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INTRODUCTION

The plight of the edematous patient with cardiac disease has for many years stimulated investigations into the underlying physiological mechanisms involved in the genesis of congestive cardiac failure. The plan of the study which we have undertaken is to follow the renal circulation and simultaneous electrolyte excretion in cardiac patients under varying conditions of rest and exercise. It is obvious that before conclusions can be drawn about cardiac patients, normal controls must first be studied. It is the purpose of this paper, therefore, to introduce our subject by presenting data obtained on normal controls.

For the past 30 years the backward pressure hypothesis of Starling (1) has dominated both the clinical and the research approach to the problem of congestive cardiac failure. In recent years, however, it has become apparent that more subtle factors must also play a part. The observation that the edematous state is poorly correlated with the intravenous hydrostatic pressure has led to a reconsideration of the problem.

Although Starling (1a) himself had commented on the impaired renal function in cardiac failure, it was not until 1942 that Schroeder and Futcher (2) focused the attention of modern investigators on the

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kidneys by showing that the cardiac patient excretes less administered salt than a normal person. Two years later Warren and Stead (3) brought forth the hypothesis that lowering of the renal blood flow and diminution of the glomerular filtration rate were the primary factors in the diminished excretion of salt seen in cardiac patients.

In 1946 Landis, Brown, Fateaux and Wise (4) in animal experiments called attention to the fact that muscular exercise may play an important role in increasing the congestive state in the presence of a damaged heart.

Although every clinician is well aware of the fact that the cardiac patient is more apt to go into congestive failure while he is at home living under the varying activities and stresses of his daily life, it is surprising to find how little experimental work has been done elucidating the role of exercise in heart failure.

At the present time it seems clear that: 1) the cardiac patient under certain conditions retains NaCl due to diminished renal excretion of sodium and chloride ions, 2) diminution of the renal blood flow and glomerular filtration rate does occur in cardiac failure, 3) muscular exercise increases the congestive state and worsens the condition of the cardiac patient. How these factors are interrelated and what is the exact renal mechanism in the genesis of congestive failure is the subject of the study which we have undertaken.

This report deals with thirteen experiments performed on three normal adult males, showing the changes which are induced both in the filtration rate and the excretion of plasma electrolytes by graded muscular exercise. Our interest has concerned the effect of mild exercise comparable to the average activity of sedentary workers and which the ambulant cardiac patient is capable of performing. The experiments described in this paper deal with no greater effort than walking at 3 mph. on a horizontal surface. The effects of change in posture and water diuresis occurring incidentally during the experiments were recorded.

METHODS

The subjects were three healthy adult males; ages 28, 30 and 33. The experiments were performed in the morning, when the subjects had eaten nothing after the evening meal of the night before. No

attempt was made to regulate the salt intake in the days prior to the experiments. It happened that one of the subjects, E. V. N., suffered a three day attack of diarrhea due to food poisoning on one occasion, and experiments were performed in this instance on the third day of the disease and on the second recovery day. Water was allowed ad libitum up to the time of the experiment. During most experiments the subjects drank 200 ml. of water after each urine collection. In several experiments all urine was collected from bedtime the night before until the end of the experiment the next morning.

The usual procedure was to have the subject void upon arrival at the laboratory in the morning. He then lay down and remained in the supine position during the control periods rising only to void. Urine collection periods varied from 10 to 30 minutes.

After two or three control periods in the supine position the test procedure was carried out. Three such procedures were used. In one, a water diuresis was induced by the ingestion of varying amounts of water, the subject remaining supine. In another, the effects of posture were studied by having the subject arise and stand quietly but not motionless for two periods. In one experiment the sitting position was tested during one collection period. Third, the effects of exercise were studied by having the subject walk on a horizontal motor-driven treadmill at the rate of 3 mph. The exercise tests were done in the winter months with the room adequately ventilated so that sweating was not perceptible. In one experiment (No. 12) performed during an attack of diarrhea, the subject walked an estimated 200 yards at about 2 mph. and examined a patient.

Venous blood samples were drawn during the control period, immediately following exercise, and at the end of the experiment. Heparin was used as the anticoagulant. In calculating the overnight clearances the blood levels obtained the following morning were used.

In those experiments in which inulin was used to measure filtration rate, 100 cc. of an isotonic 10% solution of inulin were given in a single intravenous injection over a period of 10 to 15 minutes. Twenty to thirty minutes were allowed before the first collection period was begun. Blood was drawn before the inulin injection and at the midpoint of each urine collection period.

In the treadmill experiments, in order to measure the amount of work done, the subject's expired air was collected in a Tissot gasometer analyzed for CO_2 and O_2 content by Haldane's method, and oxygen consumption was calculated. Lactic acid determinations were run on blood samples drawn before and after exercise by the method of Edwards (5).

Simultaneous determinations of filtration rate (FR), and plasma clearances of sodium (C_{Na}), potassium (C_K), chloride (C_{Cl}), and phosphate (C_{PO4}) were carried out on all urine specimens.

The FR was determined by the inulin clearance (C_{In}) method of Smith (6) in each of the subjects, and this was compared with the simultaneous endogenous creatinine clearance (C_{Cr}). Creatinine in plasma and urine was determined by the alkaline picrate method of Peters (7).

Sodium and potassium were determined on the American Cyanamide Company external standard flame photometer through the courtesy of Dr. John Eager Howard.* Chlorides were determined by the method of Keys (8) and phosphate by the method of Fiske and Subbarrow (9).

In order to avoid the injection of foreign substances which might possibly modify the electrolyte excretion pattern we have used in most experiments the endogenous creatinine clearance as the measure of glomerular filtration rate. Experiment No. 8 indicated that the injection of inulin which contains a total of 14.4 mEq. of sodium could significantly modify the pattern of sodium excretion. Following the injection of inulin the C_{Na}/C_{In} rose from control levels of 0.4% to 0.6%. The ratio C_{Cr}/C_{In} obtained by simultaneous determination of plasma clearances of inulin and endogenous creatinine was constant in each of the three subjects. Values obtained for filtration rate have not been corrected to 1.73 square meters surface area.

METHOD OF EXPRESSION OF RESULTS

The charts are constructed to yield the greatest possible amount of information concerning the relationship between glomerular filtra-

* For description of instrument see conference on metabolic aspects of convalescence, transactions of the eleventh meeting, Oct. 15–16, 1945, Josiah Macy Jr. Foundation, P9.

tion and tubular reabsorption of each substance as well as the magnitude of the change for each substance.

An important measurement in determining the total body balance of a substance is the amount of that substance excreted in the urine. This quantity can be expressed in terms of glomerular and tubular function as the proportion or percentage of the substance filtered by the glomerulus which has not been reabsorbed by the tubules. By calculating the clearance ratios of the various electrolytes studied one may obtain values for such quantities.

The clearance ratio is Clearance X/FR where X is the substance under consideration, and FR is the filtration rate whether determined by endogenous creatinine clearance (C_{Cr}) or inulin clearance (C_{In}).

The ratio of the clearance of any substance to the filtration rate is the fraction of the substance filtered which is not reabsorbed and is, therefore, excreted. The reasoning behind this statement is as follows: The clearance of a substance is expressed as the number of cc. of plasma which contains the amount of the substance which is excreted in the urine in one minute. Thus, if .140 mEq. of sodium is excreted in the urine every minute and if the plasma level of sodium is .140 mEq./cc., then the clearance of sodium is 1 cc. per minute.

Since we may assume that the concentration of sodium is the same in the plasma as it is in the glomerular filtrate we may think of the clearance of sodium as being the number of cc. of glomerular filtrate which would contain the amount of sodium excreted in one minute. If the FR is 120 cc./min. and the clearance of sodium is 1 cc./min., then the amount of sodium excreted is 1/120 of the amount of sodium filtered. This is the clearance ratio or the Clearance Sodium/FR. Expressed as a decimal this is .00833, or multiplying by 100 it becomes 0.833 per cent of the glomerular filtrate. Thus the percentage of filtered substance not reabsorbed is easily determined by the ratio of the clearance of the substance to the glomerular filtration rate. All ratios have been calculated in this way and expressed as per cent of glomerular filtrate which is excreted.

Table 1 gives the relationship, as determined by clearances, of glomerular and tubular function to NaCl balance.

Electrolyte clearance ratios have been plotted for sodium, potassium, chloride and phosphate with the ordinate on a logarithmic scale and

time as the abscissa. In order to avoid the confusion of crossing lines on the charts, each curve has been plotted separately on the same type of log paper, cut out and mounted individually. With this type of chart, if there is a greater rise or fall in the clearance ratio of one electrolyte in comparison with another, it indicates a selective change in the proportion of filtered electrolyte not reabsorbed by the tubules.

Urine concentration of any electrolyte is governed by the relative proportion of water and electrolyte filtered to that excreted. The

TABLE 1

Relationship of Clearances to Salt Balance

C_{In} or C_r FR cc./min.	C_{Na} cc./min.	$\frac{[C_{Na}/FR] \cdot 100}{\%\ \text{excreted}}$	$\frac{[1 - C_{Na}/FR] \cdot 100}{\%\ \text{reabsorbed}}$	Daily total output of Na as NaCl	Remarks
100	.5	.5	99.5	5.8 gms.	Balance
50	.5	1.0	99	5.8 gms.	Balance
100	0.25	0.25	99.75	2.9 gms.	Salt Retention
50	0.25	0.5	99.5	2.9 gms.	Salt Retention

Serum Na = 0.14 mEq./cc.

Salt intake = 5.8 gms. per day.

This table is constructed assuming a normal filtration rate of 100 cc./min. and a total intake of 5.8 gms. of salt per day. The effect of a 50% reduction of FR is also shown. It is obvious that what appear to be slight changes in per cent reabsorbed would result in large changes in salt output over a 24 hour period and that slight changes in per cent reabsorbed can compensate for marked decreases in filtration rate (FR).

proportion of filtered water excreted is the ratio Urine Flow/C_{Cr} or Urine Flow/C_{In}. The relative changes in the concentrations of electrolytes may be observed by comparing the degree of rise or fall of the clearance ratios to the ratio UF/C_{Cr}. For example a fall in electrolyte clearance ratio greater than a fall in UF/C_{Cr} means a fall in concentration of that electrolyte in the urine. Thus, with this type of chart one can observe the direction of change in urinary concentration of any electrolyte as well as the relative change of the percentage of the electrolyte filtered which is not reabsorbed by the tubules. This method of interpretation, however, does not indicate the total amount of any given electrolyte in the urine unless it should happen that the filtration rate remain constant throughout the entire experiment.

RESULTS AND DISCUSSION

In these experiments we have attempted to correlate in the normal subject the changes in urine flow, glomerular filtration rate and electrolyte excretion which are brought about by exercise. The effects of water diuresis and posture served as controls for these observations.

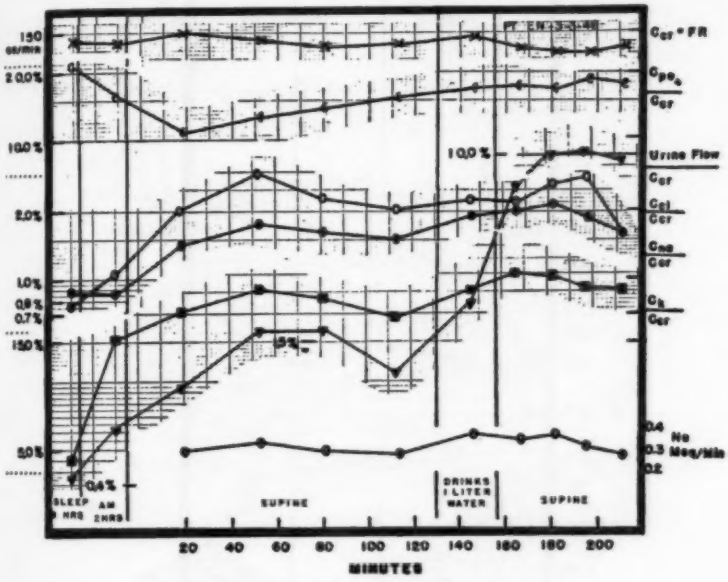
TABLE 2

Effect of Exercise on Sodium and Water Excretion

SUBJECT	EXPERIMENT	PRE-EXERCISE		EXERCISE		HIGHEST POST EXERCISE		DURATION OF C_{Na}/C_{Cr} DEPRESSION AFTER END OF EXERCISE	COMMENTS
		C_{Na}/C_{Cr}	UF/C_{Cr}	C_{Na}/C_{Cr}	UF/C_{Cr}	C_{Na}/C_{Cr}	UF/C_{Cr}		
		%	%	%	%	%	%	min.	
E. V. N.	9	1.7	9.0	.58	2.5	1.10	6.7	46	No change in FR
E. V. N.	10	1.05	6.7	.6	4.0	1.02	2.0	26	No change in FR
E. V. N.	11	.95	.43	.17	.24	.86	.90	52	Mild G-I infection. Diuresis evident toward end of experiment. Constant FR
E. V. N.	12	.26	7.4	.07	5.0	.21	6.4	40	Variation in FR 155–145–175 cc./min.
E. V. N.	13	1.44	10.6	.48	5.7	1.30	1.15	23	Constant FR
S. -S.	6	1.16	11.0	.72	7.3	1.30	9.4	30	Variations in FR 160–145–185 cc./min.
S. -S.	7	.55	6.5	.11	3.2	.46	6.0	36	Constant FR
S. -S.	8	.86	9.2	.21	3.7	.92	10.0	50	Constant FR
A. K.	5	.65	8.0	.42	5.8	.77	7.8	30	Constant FR

The most striking feature of all the experiments during mild exercise was a constant diminution in sodium excretion without a comparable change in filtration rate. Table No. 2 summarizes the essential data on the nine experiments in which the effect of exercise was tested. It is seen that C_{Na}/C_{Cr} fell consistently. The depression ranged from 17% to 65% of the pre-exercise value, average 36.4%. It was impossible to correlate the degree of depression with either the initial

control or pre-exercise value of C_{Na}/C_{Cr}. The degree of antidiuresis or the change in ratio UF/C_{Cr} had also no constant relationship

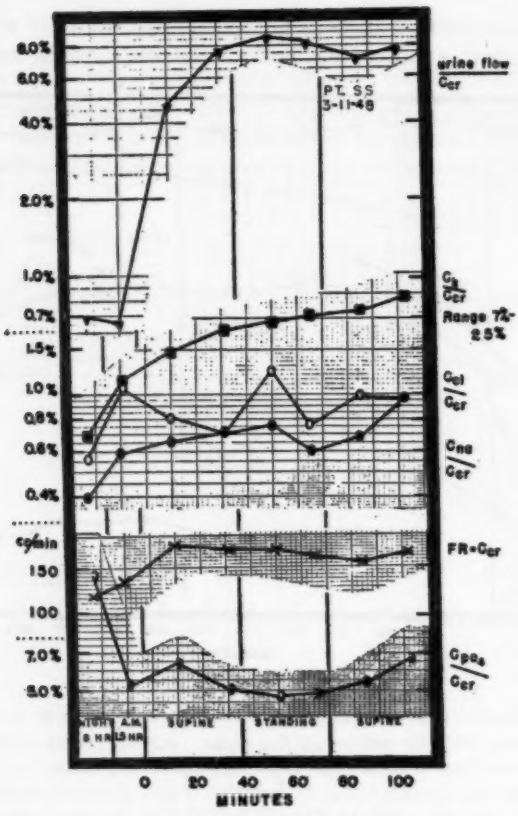


EXPERIMENT No. 1

This chart shows the effect of ingestion of one liter of water on the electrolyte clearance ratios with the subject in the supine position. After rising from bed in the morning there is an increase in all clearance ratios except for PO_4 accompanying a diuresis brought about by ingestion of two glasses of water, the usual amount taken by the subject during this period. In the three periods prior to the ingestion of one liter of water, electrolyte clearance ratios are constant. Despite the effect of diuresis (UF/C_{Cr} 1.1% to 10%) the ratios C_{PO_4}/C_{Cr}, C_{Na}/C_{Cr}, C_{Cl}/C_{Cr} show only a slight rise, C_K/C_{Cr} rising from 19.5% to 30%. The filtration rate remained constant throughout the experiment.

In this case, diuresis has only a minimal effect on the excretion of sodium, chloride and phosphate. C_{Na}/C_{Cr} is virtually unaffected by a marked change in urine volume. The absolute value for the excretion of sodium in mEq./min. shows no significant variation.

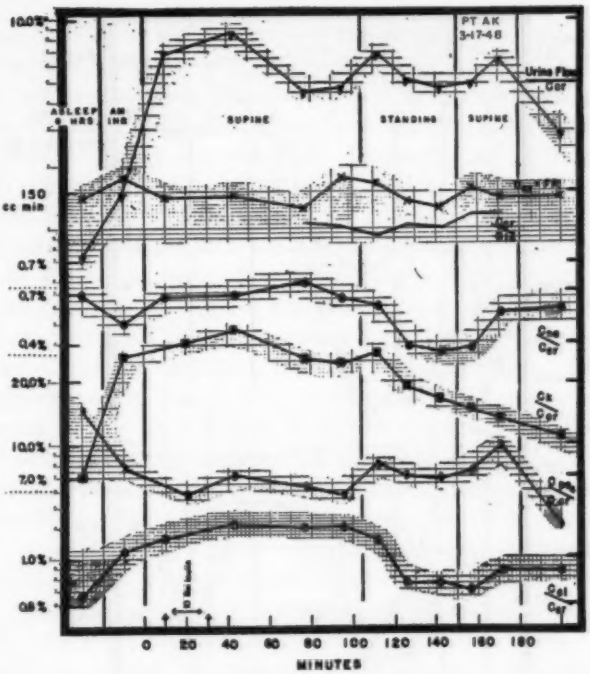
with the changes in C_{Na}/C_{Cr}. The depression of C_{Na}/C_{Cr} lasted on an average 37 minutes from the end of exercise.



EXPERIMENT No. 2

This chart shows the effect of posture (standing quietly but not motionless for 35 minutes) on the filtration rate and electrolyte clearance ratios. Water diuresis was established during the supine control period in which UF/C_{Cr} rose from .65% to 7.5%. During this time, there was a rise in filtration rate from 132 cc./min. to 185 cc./min. with a progressive increase in C_{Na}/C_{Cr} and C_K/C_{Cr}, while C_{Cl}/C_{Cr} fell from 1.05% to .7%. No significant changes in FR, C_{Na}/C_{Cr}, C_{Cl}/C_{Cr}, or UF/C_{Cr} were noted during or after the standing period.

Excretion of chloride was inhibited in all experiments as the result of exercise, following a qualitative pattern similar to that of sodium but usually to a lesser extent. This response is opposite to that

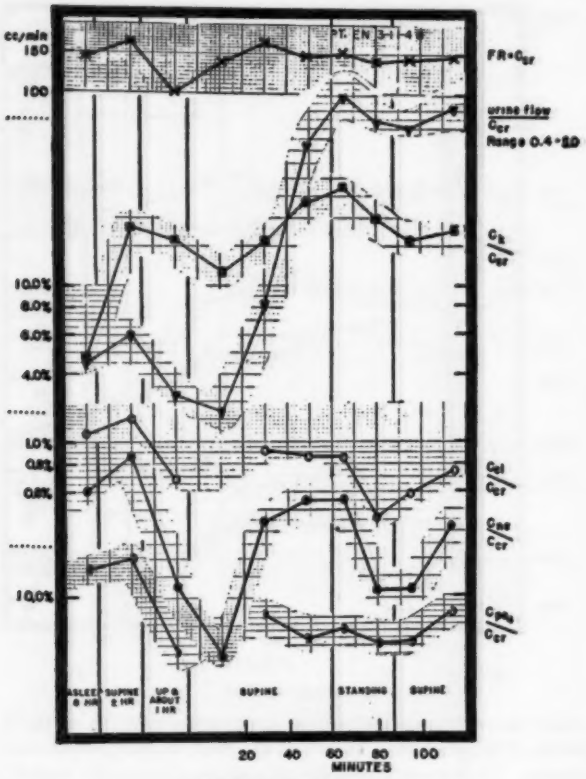


EXPERIMENT No. 3

This chart shows the comparison of inulin and creatinine methods for the determination of filtration rate, as well as the effect of erect posture for a period of 46 minutes. Diuresis was established early in the control supine position. The increase in UF/C_{Cr} from .72% to 6.8% was associated with a marked initial rise in C_K/C_{Cr}, a smaller increase in C_{Cl}/C_{Cr} and a depression of C_{Na}/C_{Cr} from .7% to .5%. The urine flow showed marked variation throughout this experiment, the depression of UF/C_{Cr} being comparable to that obtained in the control supine position. Standing in this subject produced changes of significant magnitude in C_{Na}/C_{Cr}, C_{Cl}/C_{Cr}. Variations in FR are no greater than those seen during control periods. The C_{Cr}/C_{In} shows little variation from unity.

In this experiment, the glomerular filtration rates determined simultaneously by inulin and endogenous creatinine clearance are of comparable magnitude, while this subject shows slight increased tubular reabsorption of both sodium and chloride while standing. C_{PO_4}/C_{Cr} rose during the standing period while the C_K/C_{Cr} fell progressively even during the recovery period.

The last period was taken two hours after the previous supine recovery period. During this time the subject had eaten his lunch.



EXPERIMENT NO. 4

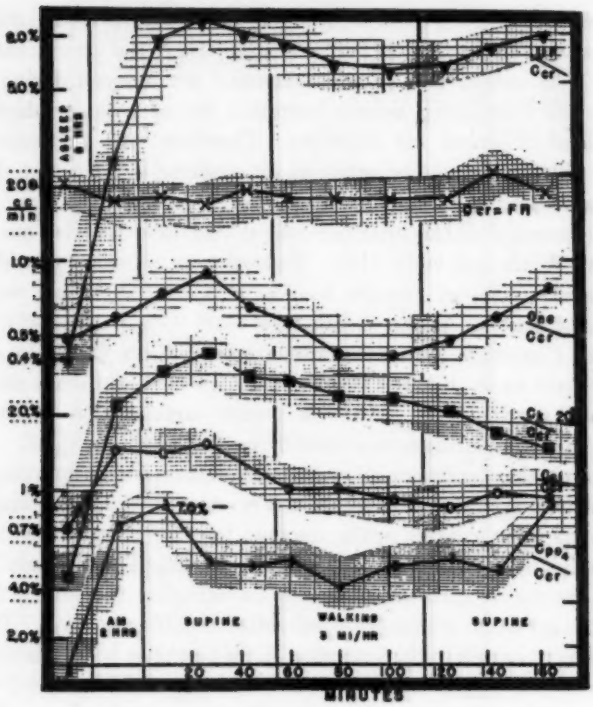
This experiment was performed two days after the subject had recovered from an episode of diarrhea. Upon awakening in the morning the patient voided and remained in bed an additional two hours, following which he walked 500 yards to the hospital. During normal activity coming to the hospital there was significant depression of C_{Na}/C_{Cr} from .85% to .23% continuing to fall to .11% during the first supine period. Diuresis was established during the second and third supine periods, UF/C_{Cr} rising from .27% to 8.0%. This was associated with a recovery of the C_{Na}/C_{Cr} ratio to its overnight level. With a change in posture there was a mild antidiuresis, the sodium and chloride ratios, falling significantly, in the presence of an unchanged filtration rate and returning to their previous values by the end of the experiment. C_{PO_4}/C_{Cr} remained constant during standing while the C_K/C_{Cr} fell from 24% to 16%. The fourth urine collection was of insufficient volume to permit analysis for Cl and PO_4.

observed by Verney (10) in pituitary antidiuresis where the chloride excretion was increased. It will be noted from the charts that although both sodium and chloride excretion are reduced during mild exercise the changes in sodium excretion are of greater magnitude than those observed for chlorides. Therefore, the depression of urinary chloride cannot be taken as an absolute index of the degree of sodium retention.

The constancy of the filtration rate during mild exercise has been noted by White and Rolfe (18). Reduction in filtration rate during more severe forms of exercise has been reported by these workers and also by Barclay, Cooke, Kenney and Nutt (19), Merrill and Cargill (20) and Chapman, Henschel, Minckler and Keys (21). In all of these reports an antidiuretic effect and a reduction of urinary chloride was noted during short bursts of severe exercise. No studies of sodium excretion during severe or mild exercise have been found.

Although the depression of sodium excretion during exercise is a constant finding, certain features of this reaction should be emphasized. First, a marked variation in the response to exercise was noted in the same individual on different occasions, as is seen in Experiments No. 7 and 8. Secondly, there were marked variations in the control levels of sodium excretion in the same individual on different days. It was impossible to correlate the intensity of the response with this control level. No correlation could be found with the initial control value or pre-exercise value of urine flow. However, on the basis of two experiments (Nos. 4 and 11) performed while the subject was having diarrhea and during the recovery stage, it appears that the state of hydration and of salt depletion may modify the degree of sodium retention due to exercise. In one of these experiments (No. 4) erect posture alone was sufficient to produce a marked depression while in another (Experiment No. 11), walking produced a similar profound effect. Thirdly, the time sequence is of some interest. In several 30 minute exercise experiments the minimal level of sodium excretion was not reached until the periods immediately following the end of exercise. In all experiments an average of 37 minutes was required before the recovery to control levels was reached after cessation of exercise.

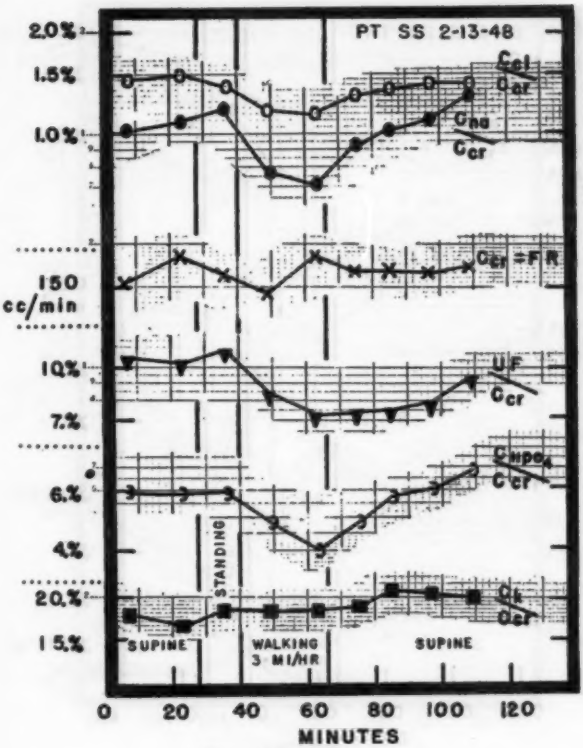
That the salt retaining response due to exercise is not a general



EXPERIMENT NO. 5

Water diuresis in this experiment was established in the control periods with the patient supine, the pre-exercise UF/C_{Cr} being 8.0%. During water diuresis there was an increase in all the electrolyte excretion ratios, the C_{Na}/C_{Cr} rising from 0.5% to 0.9%. Prior to walking for 60 minutes at a rate of 3 mph., the C_{Na}/C_{Cr} fell to .65% and exercise produced a further depression of .42%. The filtration rate remained constant throughout the experiment. The depression of sodium excretion continued for a period of 30 minutes after the end of walking. The antidiuretic effect of exercise was not marked (8.0% to 5.8%), nor was the reduction of C_{Cl}/C_{Cr}. C_K/C_{Cr} and C_{PO_4}/C_{Cr} did not change significantly during exercise.

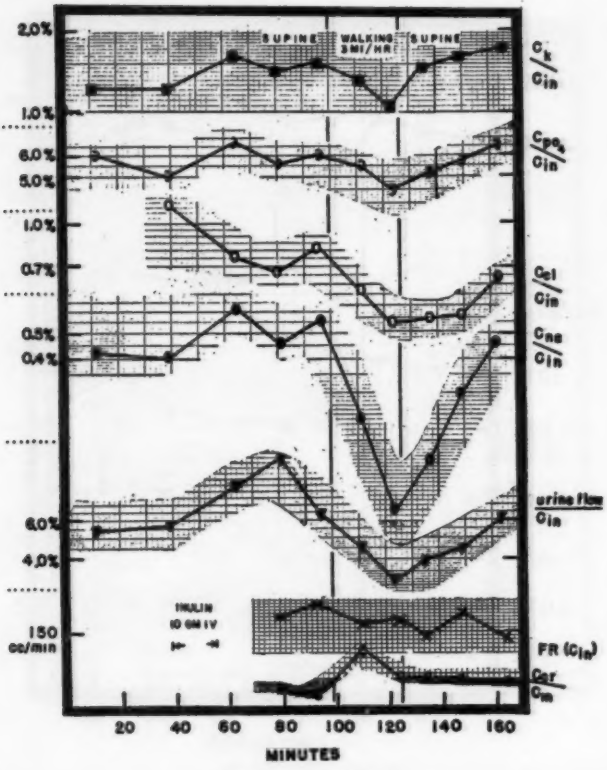
electrolyte phenomenon is shown by the clearance ratio curves of potassium and phosphate, the main intracellular ionic constituents. Although marked diurnal variation in the excretion of these elec-



EXPERIMENT No. 6

This chart shows the effect of standing for 12 minutes followed by walking at 3 mph. for 26 minutes. The UF, Na, Cl, K and PO_4 ratios were constant during the control and standing periods. The filtration rate varied from 145 to 185 cc./min. throughout the experiment. During exercise, C_{Na}/C_{Cr} fell from 1.16% to .72% returning to control levels 30 minutes after the end of exercise. A persistent antidiuresis continued until the end of the experiment. The C_{Cl}/C_{Cr} showed changes similar to C_{Na}/C_{Cr} but not of the same magnitude. It will be seen that during the second exercise period when the FR was highest the sodium excretion was at its lowest level. C_K/C_{Cr} remained constant throughout the entire experiment. C_{PO_4}/C_{Cr} fell during exercise from 6.0% to 4.0%.

trolytes is apparent, no significant variations in their excretion patterns were noted which might be attributed to posture or exercise. In some experiments they rose with exercise while in others they fell.

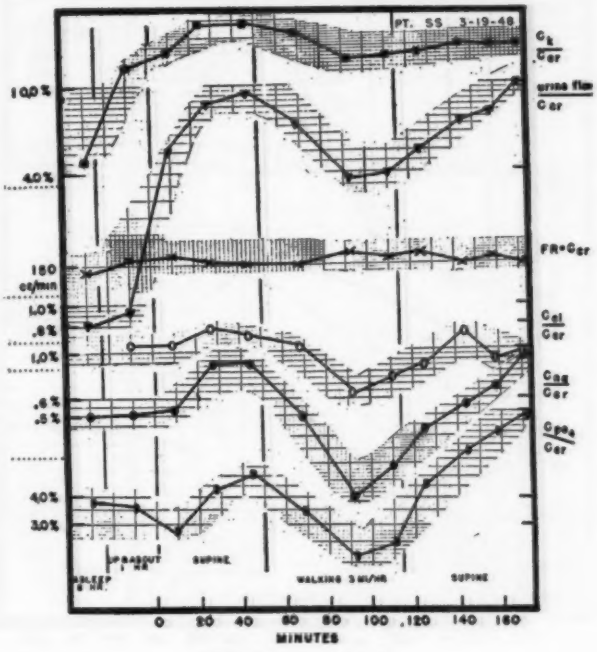


EXPERIMENT NO. 7

In this experiment, filtration rate was determined simultaneously by the use of inulin and creatinine, the average C_{Cr}/C_{In} being 1.07 and the electrolyte excretion ratios are expressed relative to the clearance of inulin. Following the injection of 10 gms. of inulin (containing 14.4 mEq. of sodium) the C_{Na}/C_{In} increased from 0.4% to 0.6%. During exercise (walking at 3 mph. for 26 minutes), the filtration rate showed no significant alteration, but C_{Na}/C_{In} fell from 0.55% to 0.11% returning only to 0.45% 30 minutes after the end of walking. Exercise produced an antidiuresis, UF/C_{In} falling from 6.5% to 3.2%, the reduction in C_{Cl}/C_{In} being of the same magnitude as the change in UF/C_{In}. Potassium and phosphate ratios showed only minor variations.

In some they remained constant. Sometimes they were parallel in their movement while at other times they moved in opposite directions.

It may be seen from the charts that the NaCl retaining response due to exercise is not due to changes in urine flow. In ten experi-

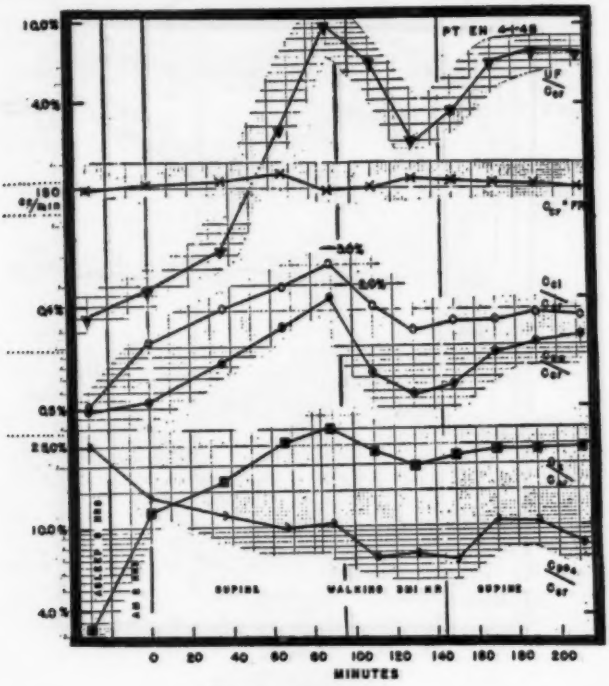


EXPERIMENT NO. 8

This chart shows the effect of walking for 64 minutes. Water diuresis was established during the control period, the UF/C_{Cr} ratio rising from an initial value of 0.8% to 9.2% prior to exercise. Associated with this diuresis, there was a marked rise in C_K/C_{Cr}, with a less marked increase in C_{Na}/C_{Cr} and C_{Cl}/C_{Cr}. The filtration rate remained constant throughout the experiment. Exercise caused an antidiuresis (9.2% to 3.7%) while C_{Na}/C_{Cr} fell from a control level of 0.86% to 0.21%, rising only to its former level 50 minutes after the end of walking. C_{Cl}/C_{Cr} showed the same trend, although the degree of depression was not so great. While C_K/C_{Cr} was very little affected by exercise, C_{PO_4}/C_{Cr} fell during exercise to about 50% of the pre-exercise level, a change of the same magnitude as UF/C_{Cr}.

ments a water diuresis was observed. In no case was any significant depression of sodium or chloride excretion observed coincident with

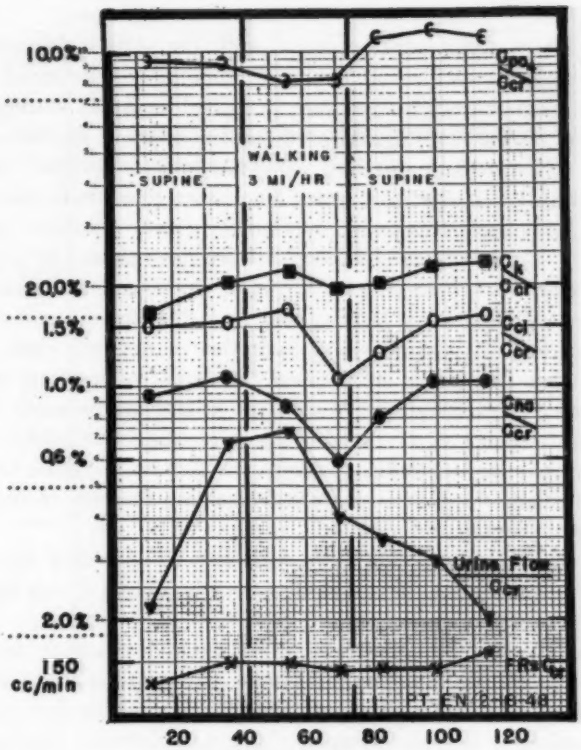
water diuresis induced by the ingestion of an excess of water. In Experiments Nos. 1, 2, 3, 5 and 9 the establishment of diuresis in the



EXPERIMENT NO. 9

This chart shows the effect of walking at 3 mph. for 60 minutes, water diuresis having been established at a UF/C_{Cr} ratio of 9.0% at the beginning of exercise. This diuresis was associated with a constant filtration rate but with an increase of the electrolyte clearance ratios, C_K/C_{Cr}, C_{Na}/C_{Cr}, C_{Cl}/C_{Cr} and a decrease in the C_{PO_4}/C_{Cr} ratio. Exercise produced no change in filtration rate, but a progressive depression of C_{Na}/C_{Cr} from 1.7% to 0.58%, the ratio returning to 1.05% 46 minutes after the patient had finished walking. This degree of exercise produced a definite antidiuresis, and at the same time, the C_{Cl}/C_{Cr} mirrored the sodium effect, the change being of small magnitude. Potassium and phosphate ratios were slightly depressed during exercise.

morning was accompanied by an increase in the sodium and chloride excretion over the overnight controls. In Experiment No. 1 a large water diuresis after the morning urine flow had been established



EXPERIMENT No. 10

Diuresis was established in the initial control periods, with the subject supine, UF/C_{Cr} rising from 2.2% to 6.7%. The electrolyte ratios C_K/C_{Cr}, C_{Cl}/C_{Cr}, C_{Na}/C_{Cr} remained constant. During 32 minutes of walking at 3 mph., the filtration rate remained constant, while an antidiuresis caused a fall of UF/C_{Cr} to 4.0%. The C_{Na}/C_{Cr} fell from 1.1 to 0.6% returning to its control level 26 minutes after the end of exercise. C_K/C_{Cr} showed little alteration. The antidiuresis continued until the end of the experiment, despite the constancy of the filtration rate, while the chloride C_{Cl}/C_{Cr} followed the sodium curve. No water was drunk after the pre-exercise control periods, showing in this instance that the depression in sodium excretion returned to control levels with a falling urine flow. The potassium and phosphate ratios showed only minor variations.

caused no significant change in the rate of sodium or chloride excretion. In Experiment No. 10 it is seen that antidiuresis itself does not cause the NaCl retaining response because a decline in urine flow due to a

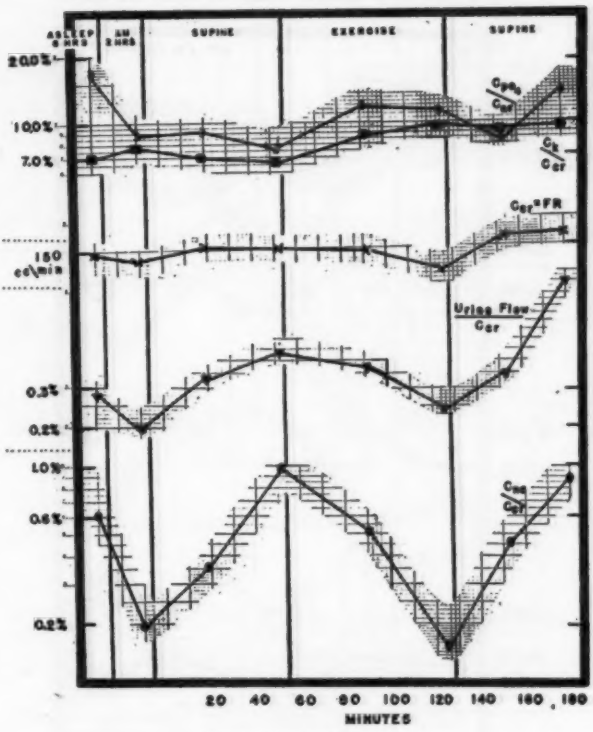
restriction of water intake occurred while the sodium clearance ratio was increasing coincident with recovery from the exercise period.

The glomerular filtration rate remained constant during water diuresis. Other investigators studying the effect of diuresis or electrolyte excretion have obtained somewhat variable results. Marshall (11) showed that during a water diuresis no measurable increase in creatinine excretion occurred but total urinary chlorides were increased. Priestley (12) noted a transitory rise followed by a progressive fall in urinary chlorides. Eggleton (13) found a diminution in chloride excretion while Barclay and Nutt (14) found marked variation, stating that the degree of hydration of the individual subject at the time of diuresis conditioned the extent of resultant chloride excretion. Crutchfield and Wood (15) in a study of water diuresis (Schemm regime) as a form of therapy for congestive cardiac failure suggest that in non-cardiac patients the sodium excretion following the ingestion of large quantities of water is a function of the initial control level of the urine flow (16).

Our results indicate that large variations in urine flow do not appreciably modify the pattern of electrolyte excretion during the short duration of these experiments.

Since the exercise experiments necessitated changing from the supine to the erect posture it became necessary to study also the changes in renal hemodynamics, urine flow and electrolyte excretion which might be brought about by changes in posture. Such changes have not been extensively investigated. Braun, Knudsen and Raaschau (17) studied changes in renal hemodynamics during passive erect posture on a tilt table. They noted a fall in the renal blood flow and filtration rate as well as an antidiuresis in normal subjects when changing from the horizontal to passive erect posture. These subjects, however, were suspended by their perineums in a saddle-like arrangement so that there was no muscular activity in the legs to preserve an adequate venous return from the lower extremities. White and Rolfe (18) observed a diminution of 8% in the filtration rate on normal subjects when changing from supine to erect posture.

We have observed no alterations either in electrolyte excretion or in renal hemodynamics when changing from the supine to the sitting posture during a 20 minute period of time (Experiment No. 13).

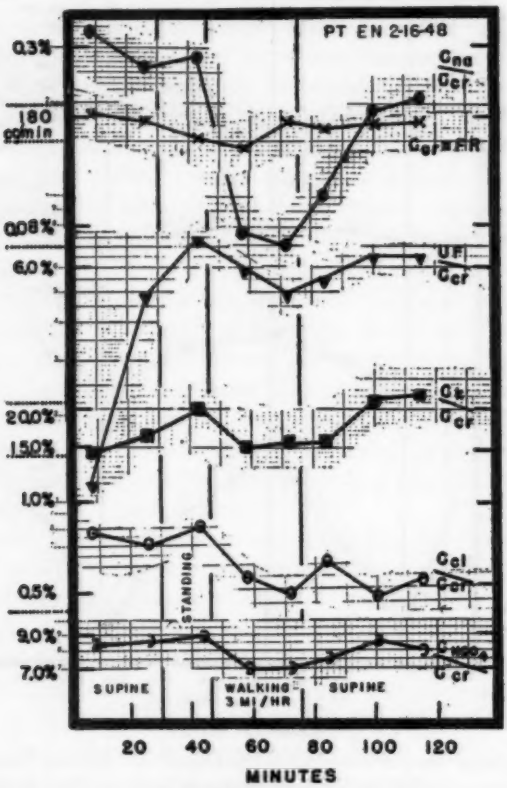


EXPERIMENT No. 11

This experiment was performed while the subject was suffering from an attack of diarrhea, the urine flow and sodium clearance being at a low level. Coming to the hospital (A.M. 2 hours) is associated with a fall of C_{Na}/C_{Cr} from 0.6% to 0.2% which returns to its overnight value during the control supine period. Exercise (walking to a consultation and back to the laboratory) caused some antidiuresis while a depression of C_{Na}/C_{Cr} is seen similar to that previously obtained. These effects return to the previous control level in the following 52 minutes.

The constancy of the filtration rate in the presence of marked variations of C_{Na}/C_{Cr} is a striking feature. The presence of a mild gastro-intestinal disturbance has, presumably, caused C_{Na}/C_{Cr} and UF/C_{Cr} to be of low value. Potassium and phosphate ratios remained constant during exercise.

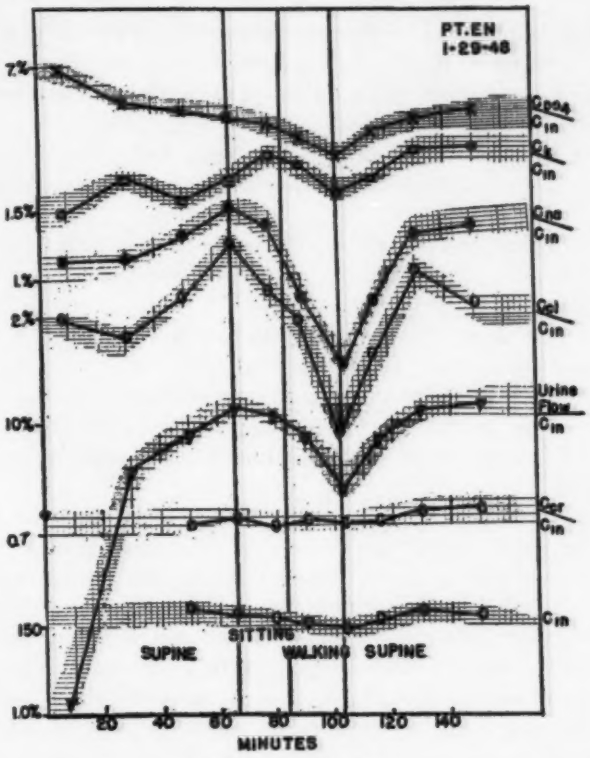
However, changing from the supine to the standing position brought about a small but definite antidiuretic effect and a small depression of sodium excretion (Experiments No. 2, 3, 4, 6 and 12).



EXPERIMENT NO. 12

This chart shows the effect of water diuresis, posture, and walking at 3 mph. for 30 minutes. During the initial 30 minutes, UF/C_{Cr} rose from 1.2% to 7.4%. The C_{Na}/C_{Cr} fell from 0.34% to 0.26% with a slight rise in C_K/C_{Cr}. During exercise C_{Na}/C_{Cr} fell from 0.28% to 0.07%, this being accompanied by a mild antidiuresis, 7.3% to 5.0%. The filtration rate showed little if any variation, while the ratio C_{Na}/C_{Cr} had not returned to the control level by the end of the experiment, 40 minutes after the end of exercise. Slight depression occurred in the potassium and phosphate ratios comparable in magnitude to the depression in UF/C_{Cr}.

Oxygen consumption was determined during exercise in the experiments and corrected to 1.73 Sq. M. of surface area. Each subject



EXPERIMENT NO. 13

Diuresis was established during the supine control period UF/C_{Cr} rising from 1.1% to 11.0%. During this time, the ratios of C_{Cl}/C_{In}, C_K/C_{In} and C_{Na}/C_{In} increased to some degree, and the effects produced by a change of posture are further accentuated by walking for a period of 20 minutes at 3 mph. An antidiuresis occurred, UF/C_{In} falling from 1.1% to 0.8%. The C_{Na}/C_{In} and C_{Cl}/C_{In} ratios showed a marked depression, C_{Na}/C_{In} falling from 1.7% to 0.48% nearly a four fold change. Filtration rate fell from 160 to 150 during exercise. The ratio C_{Cr}/C_{In}, although not unity, showed constancy through the experiment, ranging from 0.7 to 0.8. The potassium and phosphate ratios showed slight depression during exercise.

showed marked constancy; the range for 19 determinations being 0.875 to 1.18 with an average of 1.07 l./min./1.73 Sq. M. body surface area.

Two determinations of oxygen consumption were made on each subject, one during the first half and the other during the second half of the exercise periods. The values were constant for each subject throughout the excercise period.

Blood lactic acid levels determined during and immediately after exercise showed no significant variation from the pre-exercise control level.

Serum sodium and potassium levels were taken in each experiment before and after exercise and in each instance they remained constant within the limits of error in the method.

Three possible mechanisms by which this NaCl retaining response might be mediated have been considered.

1) The possibility that there was increased reabsorption of sodium to compensate for that lost by sweating has been minimized by conducting the experiments under conditions where sweating was not perceptible.

2) Substances liberated from the tissues during exercise might affect the renal tubule. An increase in blood lactic acid is not responsible, and we assume that anaerobic muscle work is not necessary to elicit this renal tubular rentention of sodium.

3) Hormonal influences either of the adrenal cortex or posterior pituitary may mediate this response. If the specific tubular retention of sodium which occurs during exercise can be regarded as an adaptation phenomenon, further experiments should be done to determine what physiological function this effect serves. At this stage we feel justified only in stating that mild exercise which produces no measurable change in glomerular filtration rate evokes a NaCl retaining response. The mechanism of this phenomenon will be the subject of further study in normal subjects. Its implication in the genesis of congestive cardiac failure will be the subject of a further report.

CONCLUSIONS

1) Thirteen experiments on three normal adult males have been reported to show the effect of standing and walking on the glomerular filtration rate and the renal mechanism of sodium, chloride, potassium, phosphate and water excretion.

2) The exercise was walking at 3.0 mph. on a motor driven tread-

mill. The average oxygen consumption during this work was 1.07 l./min./1.73 Sq. M. body surface area (range 0.88–1.18). There was no increase in blood lactic acid due to this work.

3) This exercise produced no significant alteration in glomerular filtration rate or serum sodium and potassium concentrations.

4) Exercise caused a specific sodium and chloride retention due to a decrease in the proportion of filtered sodium excreted or an increase in the tubular reabsorption of filtered sodium. The retention persisted an average of 37 minutes after the end of exercise.

5) Standing quietly has a slight sodium and chloride retaining effect.

6) A variable antidiuresis occurred with exercise.

7) The potassium and phosphate excretion did not show any predictable correlation with the changes in sodium excretion.

Acknowledgements: The authors are indebted to Dr. E. K. Marshall, Professor of Pharmacology, Drs. E. Cowles Andrus and John Eager Howard, Associate Professors of Medicine and Mr. Harry Eisenberg, Chemist, The Johns Hopkins Hospital for advice and assistance given for theoretical and metabolic problems encountered in this study.

BIBLIOGRAPHY

1. STARLING, E. H., The Linacre lecture on the law of the heart. London, Longmans, Green, 1918.
1a. STARLING, E. H., Physiological factors involved in the causation of dropsy. Lancet, **1**, 1267, 1896.
2. FUTCHER, P. H. AND SCHROEDER, M. A., Studies on congestive heart failure. II. Impaired renal excretion of sodium chloride. Am. J. Med. Sci., **52**, 204, 1942.
3. WARREN, J. V. and STEAD, E., Fluid dynamics in chronic congestive heart failure, interpretation of mechanism producing edema, increased plasma volume and elevated venous pressure in certain patients with prolonged congestive failure. Arch. Int. Med., **73**, 138, 1944.
4. LANDIS, E. M., BROWN, E., FATEAUX, M. AND WISE, C., Central venous pressure in relation to cardiac "competence", blood volume and exercise. J. Clin. Invest., **25**, 237, 1946.
5. EDWARDS, H. T., A simplified estimation of lactate in normal human blood. J. Biol. Chem., **125**, 571, 1938.
6. GOLDRING, W. AND CHASIS, H., Hypertension and hypertensive disease. The Commonwealth Fund, New York, 1944.

7. PETERS, J. H., The determination of creatine and creatinine in blood and urine with the photoelectric colorimeter. J. Biol. Chem., **146,** 179, 1942.
8. KEYS, A., The microdetermination of chlorides in biological materials. J. Biol. Chem., **119,** 389, 1937.
9. FISKE, C. H. AND SUBBARROW, Y., The colorimetric determination of phosphorus. J. Biol. Chem., **66,** 375, 1925.
10. VERNEY, E. B., The secretion of pituitrin in mammals as shown by perfusion of the isolated kidney of the dog. Proc. Royal Soc., 13, **99,** 487, 1926.
11. MARSHALL, E. K., The influence of diuresis on the elimination of urea, creatinine and chlorides. J. Pharm. & Exp. Therap., **16,** 141, 1920.
12. PRIESTLEY, J. G., Regulation of excretion of water by the kidney. J. Physiol., **55,** 305, 1921.
13. EGGLETON, M. G., Chloride excretion during water diuresis and the effects of exercise. J. Physiol., **102,** 140, 1943.
14. BARCLAY, J. A. AND NUTT, M. E., Urinary changes during diuresis. J. Physiol., **103,** 20, 1944.
15. CRUTCHFIELD, A. J. AND WOOD, J. E., Urine volume and total renal sodium excretion during water diuresis. Ann. Int. Med., **28,** 1, 28, 1948.
16. SCHEMM, F. R., High fluid intake in management of edema. Ann. Int. Med., **17,** 952, 1942.
17. BRAUN, C., KNUDSEN, E. O. AND RAASCHAU, F., The influence of posture on kidney function. Acta. Med. Scan., **122,** 315, 1945.
18. WHITE, H. S. AND ROLFE, D., Effects of exercise and of some other influences on the renal circulation in man. Am. J. Physiol., **152,** 505, 1948.
19. BARCLAY, J. A., COOKE, W. T., KENNEY, R. A. AND NUTT, M. E., The effects of water diuresis and exercise on the volume and composition of the urine. Am. J. Physiol., **148,** 2, 327, 1948.
20. MERRILL, A. J. AND CARGILL, W. H., The effect of exercise on the renal plasma flow and filtration rate of normal and cardiac patients. J. Clin. Invest., **27,** 272, 1948.
21. CHAPMAN, C. B., HENSCHEL, A., MINCKLER, J. AND KEYS, A., Effect of exercise on renal plasma flow. Fed. Proc., **7,** 1, pt. 1, 20, 1948.

THE RENAL MECHANISM OF ELECTROLYTE EXCRETION AND THE METABOLIC BALANCES OF ELECTROLYTES AND NITROGEN IN CONGESTIVE CARDIAC FAILURE; THE EFFECTS OF EXERCISE, REST AND AMINOPHYLLIN[1]

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INTRODUCTION

The increase in extracellular fluid which occurs in congestive cardiac failure has emphasized the importance of sodium excretion in this condition. This paper reports observations on three cases of congestive cardiac failure and will indicate the influence which the renal tubules exert on the excretion of the main extracellular fluid cation.

The mechanism of edema has been explained on the basis of altered hydrostatic and osmotic relationships in the peripheral vessels and the appearance of edema in cardiac decompensation has been accepted as a clinical sign of myocardial failure (1) (2) (3) (4) (5) (6). The knowledge that sodium excretion is impaired in congestive failure (7) (8) (9) has suggested that the hypervolaemia of this condition may, in part, result from abnormal renal function (10). Diminished renal blood flow and glomerular filtration rate, with a high filtration fraction, has been found to occur during cardiac decompensation (11). These circulatory changes within the kidney have been accepted as causing the abnormal retention of salt and water whereby the amount of sodium conveyed to the kidney for excretion is diminished—"The decreased excretion rate of sodium in congestive cardiac failure is attributable to a decreased filtration rate in the presence of a normal tubular mechanism" (12). The observations reported in this paper

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suggest that the increased reabsorption of filtered sodium by the renal tubules may play a part in the accumulation of extracellular sodium, equally important as that due to a decrease in the absolute amount of sodium which is filtered by the glomeruli.

Aminophyllin and mild muscular exercise produce opposing effects on the excretion of sodium by their action on the renal tubules (13) (14). Mild muscular exercise in normal subjects causes, in the presence of an unchanging filtration rate, a specific sodium retaining response. Aminophyllin, in the absence of changes in the renal circulation, produces decreased tubular reabsorption of sodium and chloride. This paper will report (1) the effects of aminophyllin and mild muscular exercise in one case of congestive cardiac failure while on metabolic balance studies and (2) the effect of mild muscular exercise on two other cases of congestive cardiac failure.

CASE REPORTS

Patient No. 1, L. S., colored female, age 38 years, surface area 1.62 sq. m.

Admitted to the Medical Service of The Johns Hopkins Hospital on November 7, 1947 in her first attack of congestive cardiac failure due to rheumatic mitral stenosis and aortic insufficiency. Marked peripheral edema was present. Blood pressure, 146 systolic, 76 diastolic mm. Hg, weight 78 kg., venous pressure 196 mm. water. There was a history of intermittent digitalis therapy over the preceding three months with no dietary salt restriction. Observations on this patient are divided into three periods.

Period No. 1 (21 days) November 7, 1947 to November 28, 1947. Experiments No. 1, 2 and 3 were performed during this period. Prior to Experiment No. 2 the patient had been digitalized and had received two injections of 2 cc. of mercupurin. All diuretics were discontinued from this time. Maintenance digitalis 0.1 gm. daily was commenced and continued throughout this study. At the end of this period, some pitting edema of the ankles still existed and the venous pressure was 100 mm. water. Diet during this period contained 2 gm. salt.

Period No. 2 (24 days) November 28, 1947 to December 22, 1947. Patient was transferred to Metabolic Ward of the Medical Division and placed on balance studies for nitrogen, sodium, potassium and chloride. Maintenance digitalis 0.1 gm. daily was given, no diuretics being used. From Day 1–15 diet contained 1 gm. salt. From Day 16–23 an additional 5 gm. of salt were added to the food. Experiments No. 4, 5 and 6 were performed on Days No. 7, 12 and 21. By the end of this period, the patient was edema-free, weight was 60.0 kg. and the venous pressure was 10 mm. water.

Period No. 3 (92 days) December 22, 1947 to March 23, 1948. Patient followed in Out-Patient Department. Therapy consisted of 0.1 gm. digitalis daily, no

diuretics were used and a voluntary salt restriction of 2 gm. daily was attempted. Clinical evidence of congestive failure with edema was progressive from the end of January, 1948. By February 27, 1948, weight was 73 kg., venous pressure 115 mm. water and ankle edema was present. Experiments No. 7, 8 and 9 were performed on Days No. 67, 74 and 92.

Patient No. 2, L. H., white male, age 50 years, surface area 1.68 sq. m.

Patient admitted to the Medical Service of The Johns Hopkins Hospital on April 15, 1948 in his first attack of congestive cardiac failure which had developed following an anterior myocardial infarction six weeks previously. The patient had been digitalized two weeks before admission and 0.1 gm. of digitalis daily was continued while in the hospital. No diuretic drugs were given. On admission weight was 63 kg., peripheral venous pressure 87 mm. water, blood pressure 118 systolic, 74 diastolic mm. Hg. Experiment No. 10 was performed on April 20, 1948 at which time the patient was dyspneic on exertion, paroxysmal nocturnal dyspnea was present, and 1+ ankle edema existed.

Patient No. 3, B. M., white male, age 30 years, surface area 1.91 sq. m.

Patient admitted to the Medical Service of The Johns Hopkins Hospital December 11, 1948 with a history of recurrent attacks of exertional dyspnea, orthopnea, abdominal swelling and ankle edema of one and a half years duration. Patient had suffered from rheumatic fever at the age of 11 and 18 years. Main physical findings at the time of admission included auricular fibrillation (apical rate 72/min. with pulse deficit 23/min.) blood pressure 110 systolic mm. Hg, cardiac enlargement with apical impulse in the fifth left intercostal space 11 cm. from the midsternal line with a mid diastolic murmur heard within the area of the apex beat. The liver was enlarged 8 cm. below the right costal margin and minimal ankle edema was present. At the time of admission the patient was receiving digitoxin 0.1 mgm. daily and no diuretics had been used for a preceding two weeks. Experiment No. 11 was performed on December 15, 1948, at which time the patient was markedly dyspneic on exertion.

METHODS

The methods used in these studies have been previously described (15). Filtration rate was determined either by the inulin or endogenous creatinine clearance methods. Renal plasma flow was measured by the clearance of para-acetylaminohippuric acid (PACA) on a falling plasma concentration curve after a single intravenous injection. Electrolyte excretion has been expressed either by clearance or clearance ratios. Clearance ratios are used in order concisely to represent the changes in urinary electrolytes in terms of the relationship bebetween the amount filtered through the glomeruli and the amount reabsorbed by the tubules. The expression 'clearance ratio' used in

this paper means the percentage of filtered electrolyte not reabsorbed by the renal tubules. This expression is : $\frac{C_{(electrolyte)}}{C_{(inulin)} \text{ or } C_{(creatinine)}} \times 100.$

If a fall in filtration rate below normal occurs with a constant clearance ratio, the decrease in quantity of electrolyte in the urine will be proportional to the decrease in filtration rate. Decreases in electrolyte output are then due primarily to a fall in filtration rate without a change in the relationship of renal tubular reabsorption to filtration rate. If there is an increase in the tubular reabsorption of electrolyte above the proportion previously or normally found, the ratio will fall, whether there has been a fall in filtration rate or not.

TABLE 1

Patient L. S. Renal Hemodynamic Changes and Blood Chemical Findings

DAY	EXPERIMENT	FILTRATION RATE	RENAL PLASMA FLOW	FILTRATION FRACTION	PLASMA Na mEq./l.	PLASMA K mEq./l.	PLASMA Cl mEq./l.	PLASMA PROTEINS			N.P.N.	SERUM PHOSPHORUS	VENOUS* PRESSURE mm. H_2O
								Tot.	Alb.	Glob.			
Period No. 1 (11/7/47–11/28/47). 21 Day Period of Cardiac Compensation													
		cc./min.	*cc./min.*					*gm. %*	*gm. %*	*gm. %*	*mg. %*	*mg. %*	
1	1	101	187.5	.54	138	4.2	115	6.4	3.1	3.3	28	4.8	196
5	2	106	269	.39	135	4.0	107	7.5	3.8	3.7	26		130
14	3	117	267	.44	136	4.6	113	7.3	4.0	3.3	30	4.3	100
Period No. 2 (11/28/47–12/22/47). 24 Day Period of Metabolic Balance Study													
1					138	4.2	104	7.6	3.9	3.7	36		
4		85	267	.40	140	5.1	115.8				26		70
7	4	108	347	.31			110						50
12	5	94	302	.31	139	4.3	106	7.3	4.0	3.3	30		25
21	6	99.2	417	.23	148	4.5	115	7.9	3.8	4.1	31		10
Period No. 3 (12/22/47–3/23/48). 92 Day Period of Cardiac Decompensation													
67	7	127	255	.49	144	3.4	115					4.3	115
74	8	94			131	4.0	112					4.1	185
92	9	100			135	4.2	103	7.1	3.9	3.2	29	5.1	118

* All venous pressure for this patient were measured from a constant arbitary zero point, the patient being elevated at 40° to the horizontal.

Renal Function during Cardiac Compensation

Table 1 shows relevant data for the Periods No. 1, 2 and 3 throughout the study of Patient No. 1, L. S.

Period No. 1: Glomerular filtration rate was not significantly depressed during the acute stage of decompensation (normal range for this patient 109± 14.6 cc./min.), although renal plasma flow was low with a filtration fraction of 0.54. During the period of digitalis administration, there was a progressive rise in renal plasma flow with a resultant fall in the filtration fraction. The sodium clearance when determined on the first hospital day at bed rest was 1.02 cc./min. Plasma sodium levels varied between 136–138 mEq. Na/l. Peripheral venous pressure fell from 196 to 100 mm. water.

Period No. 2 shows similar changes occurring during the period in which the patient was followed on metabolic balance studies. On Day 21 the renal plasma flow was 417 cc./min. with a filtration fraction of 0.23. Plasma sodium levels were within normal range (138–148 mEq. Na/l.). Following the addition of an extra 5 gm. of sodium chloride to the diet on Day No. 16, the patient at this stage of cardiac compensation was able to excrete all of the extra amount of sodium.

Period No. 3 presents data during the out-patient observation of this patient when her weight had risen from 60 to 73 kg. and her venous pressure from 10 to 118 mm. water. Filtration rate varied between 94–127 cc./min. but in one determination made on renal plasma flow, the filtration fraction had risen to 0.49. Plasma sodium concentration (131–135 mEq. Na/l.) were at the lower limits of normal. Sodium clearances diminished from 1.0 to 0.23 cc./min.

Metabolic Distrubances Observed during Cardiac Failure

The results obtained during Period No. 2 (Patient L. S.) are represented in chart No. 1 by the method advocated by Albright (16). Significant findings are:

1. The initial positive nitrogen balance persisted until the 19th day and averaged 3 gm. nitrogen/day.

2. Plasma albumin and globulin concentrations were within normal range.

3. Positive potassium balance occurred during the period of positive nitrogen balance.

4. Negative sodium and chloride balance existed until the end of the 15th day and was at that time, of the order of 40 mEq./day. On the 16th day an additional 5 gm. of salt were added to the diet and positive sodium and chloride balance occurred on the 16th and 17th

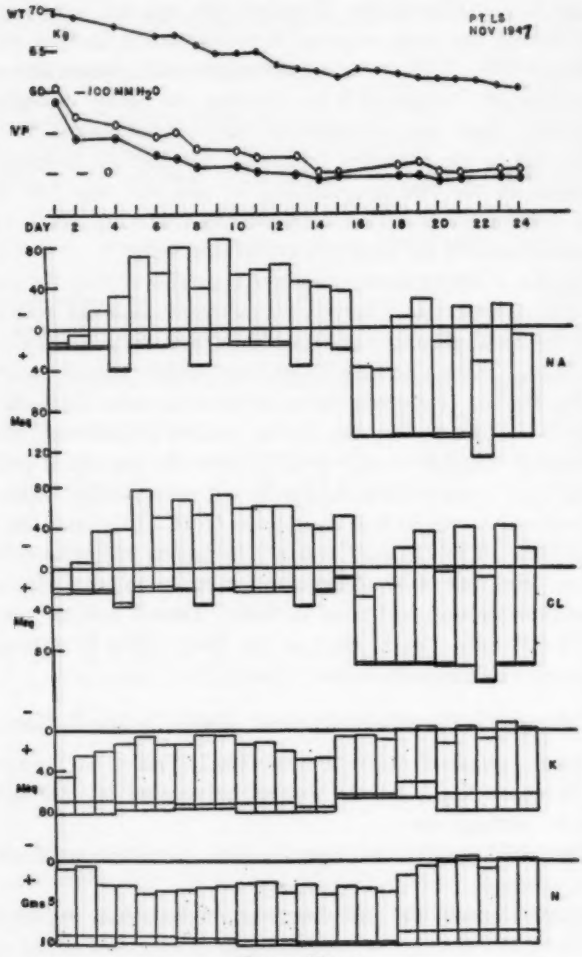

CHART NO. 1

METABOLIC BALANCE DATA OBTAINED FROM PATIENT WITH CONGESTIVE CARDIAC FAILURE

Patient No. 1: L. S. (Period No. II)

This chart shows changes in weight, venous pressure and balance data for sodium, chloride, potassium and nitrogen during Period No. II. The scale for

day. Thereafter until the end of the experimental period, sodium and chloride equilibrium existed, the extra salt being all excreted. Filtration rate was 99.2 cc./min., and the sodium clearance was 1.07 cc./min. At this point, the patient was almost edema-free.

5. Weight decreased progressively from 69.5 kg. to 60.0 kg.

Although hypoproteinemia was not present in this case, positive nitrogen balance was present from the first to the 19th day of the observation period. During the period of recompensation, negative sodium and chloride balance existed, the positive potassium balance closely following that of nitrogen. Assuming that 2.7 mEq. of potassium is associated with 1 gm. of nitrogen in protoplasm (16) (17) and deriving the amount of potassium added to intracellular fluid in protoplasm from the nitrogen balance, all of the retained potassium cound not be accounted for on the basis of protoplasm synthesis. It would be interesting to know whether changes in the base-binding power of protoplasm undergo alteration in cases of congestive cardiac failure with sodium in part replacing protassium as the main intracellular cation.

Action of Aminophyllin in Congestive Cardiac Failure

Experiments No. 1–3 demonstrate the dual action of aminophyllin as a diuretic in congestive cardiac failure. In Experiment No. 1 when the patient was in marked failure and not completely digitalized, aminophyllin 0.24 gm. administered intravenously caused lowering of the venous pressure, increased renal blood flow and glomerular filtration rate, probably secondary to increased cardiac output. How-

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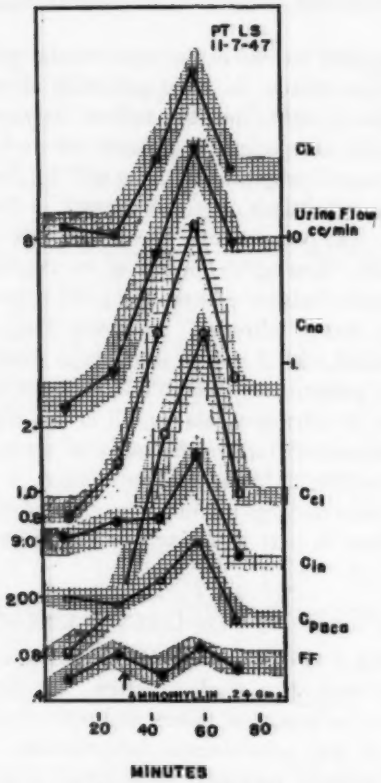
intake and balance in gm. or mEq./24 hr. is given as the ordinate; time is expressed in days as the abscissa. The horizontal line at 0 of the ordinate is the base line to which intake and balance refer. Intake is plotted as an area from the base line toward the bottom of the diagram; excretion is represented by a horizontal line measured from the bottom of the intake line. The upper line for venous pressure is in the supine position, the lower for a standard 40° vertical position. During this period weight fell from 69.5 kg. to 60.0 kg., venous pressure (supine) from 100 to 10 mm. water. From Day 1–15 negative sodium and chloride balance existed. From the 15th day an extra 5 gm. of salt was added to the diet. This was all excreted by the patient in the subsequent days. From Day 1–19 positive nitrogen and potassium balance occurred concurrently; the positive nitrogen balance being approximately 3 gm. nitrogen per day.



EXPERIMENT No. 1

Patient No. 1: L. S.

In this experiment, filtration rate, renal plasma flow and simultaneous clearances of sodium, chloride and potassium are shown for two periods before and three after the intravenous administration of 0.24 gm. aminophyllin. In the first collection period following the injection, there is approximately a three-fold increase in sodium, chloride and water excretion with little change in filtration rate or renal plasma flow. In the next collection period, there is a two-fold rise in renal plasma flow and filtration rate. At the same time, the sodium and water excretion is increased seven-fold and the chloride excretion ten-fold above the pre-injection control level. In the last period the renal blood flow and glomerular filtration rate have fallen to their control values, but the sodium and chloride excretion and urine flow still remain elevated. Following the injection of aminophyllin, the venous pressure fell from 196 to 150 mm. water.

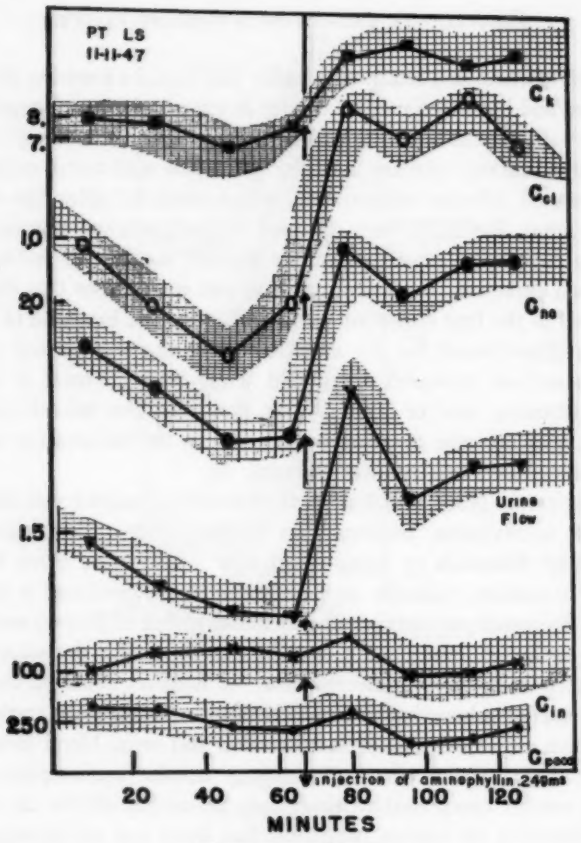
Electrolyte excretion is expressed as plasma clearance (cc. per minute).

arth, McMichael and Sharpey-Schaffer (18) found a lowering of venous pressure and increased cardiac output in experiments performed under similar circumstances. Superimposed on the circulatory effects, there was marked increase in sodium, chloride and water output due to decreased tubular reabsorption, which persisted after the changes in the renal circulation had returned to their original normal value. It is interesting to observe that the tubular mechanism initiates the increased excretion of sodium, chloride and water since this change is observed in the first collection period following the injection of aminophyllin (Experiment No. 1), at a time when the sodium and chloride clearances had increased three fold while little increase is seen in either filtration rate or renal plasma flow. In the second collection period, this increase in urinary excretion is the summation of both circulatory and renal tubular changes.

Experiments No. 2 and 3 show that when the patient was fully digitalized, intravenous aminophyllin caused little or no change in glomerular filtration or renal blood flow. The same three fold increase in sodium, chloride and water excretion produced a diuresis, due to depressed percentage tubular reabsorption of filtered sodium.

The action of aminophyllin as a diuretic in cardiac decompensation apparently is due to two mechanisms: (1) A direct effect on the circulation, causing a lowering of venous pressure and increased cardiac output which augments glomerular filtration and renal blood flow, (2) a specific renal tubular effect, diminishing tubular reabsorption. Following cardiac compensation there may be cardiac effects (as seen by slight lowering of venous pressure), but there are no corresponding changes in the renal circulation, and the diuresis which ensues is due solely to the action of the drug on the renal tubules.

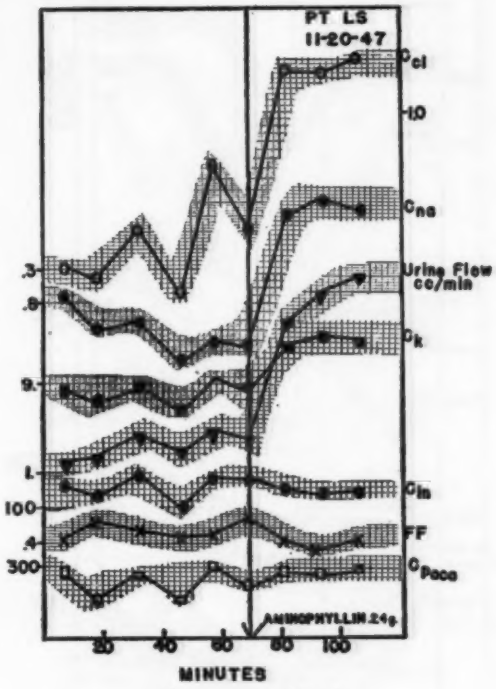
From these experiments it would appear that the mechanism of action of therapeutic doses of aminophyllin cannot be assessed without consideration of the changes in the physiological characteristics of the patient's disease. The effect of the drug may be upon the whole cardiovascular system as well as directly upon the kidney. The size of the dose and the route and speed of administration may also be important factors in determining the magnitude of the response and whether the predominant response is either cardiac or renal in origin.



EXPERIMENT NO. 2

Patient No. 1: L. S.

Experiment performed after four days of treatment. Four control periods were obtained before the intravenous administration of 0.24 gm. aminophyllin. In contrast to Experiment No. 1, there are no significant changes in renal hemodynamics following the injection and no alteration in venous pressure occurred. However, the sodium and chloride excretion and urine flow show three-fold increases, a change which continues until the end of the experiment. The magnitude of the increase is interesting in that it corresponds to that seen in the first collection period after the injection of aminophyllin in Experiment No. 1. The action of aminophyllin in this instance was entirely on the renal tubular mechanism, causing no alteration in renal hemodynamics. Venous pressure at beginning of experiment was 130 mm. water. Electrolyte excretion is expressed as plasma clearance (cc. per minute).



EXPERIMENT No. 3

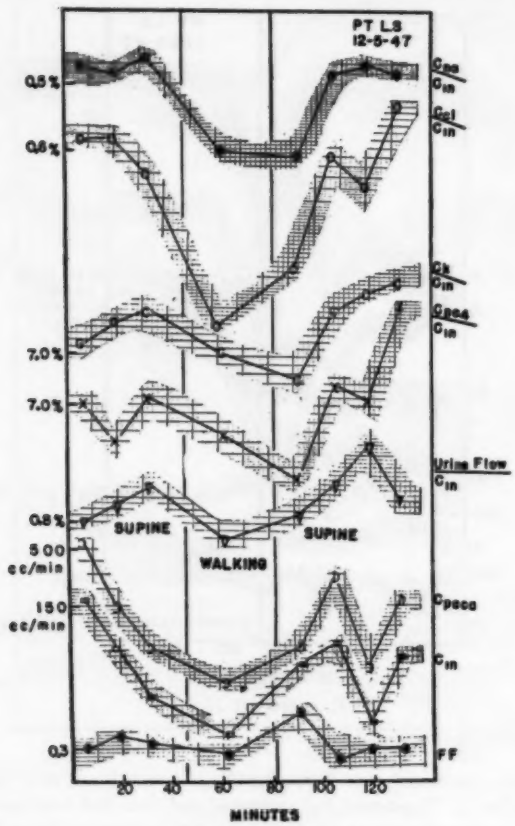
Patient No. 1: L. S.

Experiment performed nine days after Experiment No. 2 and 13 days after Experiment No. 1. Treatment had continued and there had been further clinical improvement although patient still had some pitting edema of the ankles and the liver was still enlarged. Venous pressure was 100 mm. water. Six control observations were made before injection. Results obtained in this experiment are similar to those in Experiment No. 2. No renal hemodynamic changes occurred following the intravenous administration of 0.24 gm. aminophyllin, and the same threefold increase in sodium, chloride excretion and urine flow occurred. Following the injection, the venous pressure fell to 65 mm. water.

Electrolyte excretion is expressed as plasma clearance (cc. per minute).

Effect of Exercise in Congestive Cardiac Failure

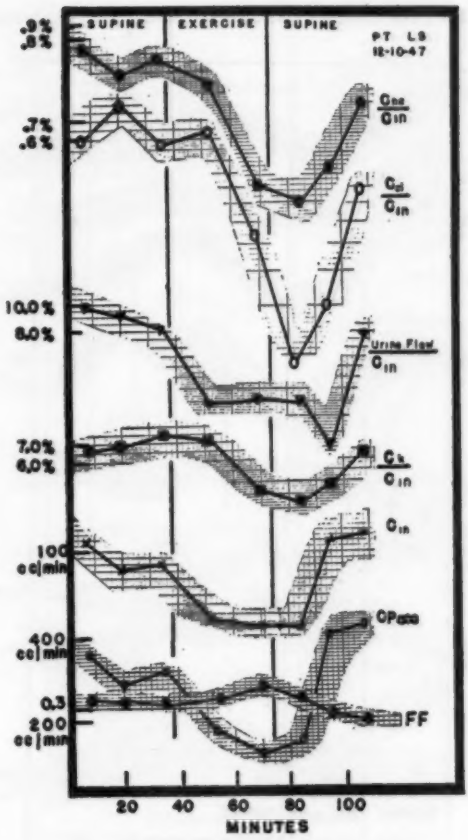
Experiments No. 4–11 show the changes in electrolyte excretion during mild muscular exercise in Patients No. 1, 2 and 3. In all ex-



EXPERIMENT NO. 4

Patient No. 1: L. S.

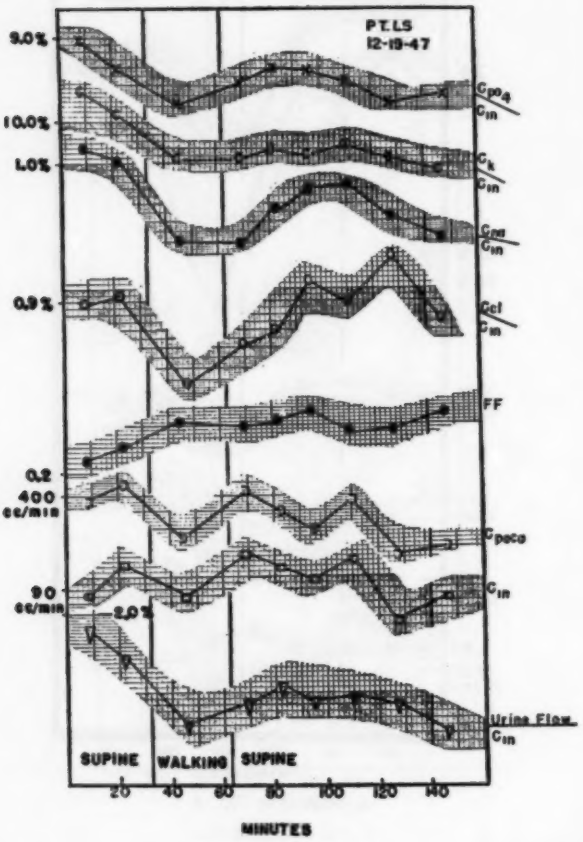
Three control periods in the supine position demonstrate a marked downward trend in the figures for filtration rate and renal plasma, making deductions concerning the effect of exercise on these functions invalid. This was thought to be due to faulty urine collection. The electrolyte excretion ratios are constant prior to exercise, the fluctuations produced by faulty urine collection being eliminated by the method of representation. Exercise (intermittent walking 300 yards in 35 minutes) caused a marked depression in the urinary excretion of chloride and sodium, the latter effect continuing for 23 minutes before C_{Na}/C_{In} returned to its pre-exercise control level. Venous pressure before exercise was 50 mm. water; after exercise, 45 mm. water.



EXPERIMENT NO. 5

Patient No. 1: L. S.

The three control periods prior to exercise show a constant filtration rate and renal plasma flow. Exercise in this experiment consisted of 35 minutes intermittent walking along the ward corridor (350 yards). In the first exercise period, an equivalent reduction in renal plasma flow and filtration rate occurs (constant filtration fraction) while the ratios C_{Na}/C_{In} and C_{Cl}/C_{In} show no great change. By the end of the exercise period there is further reduction in filtration rate and renal plasma flow; the maximal depression of the sodium and chloride ratios being in the first post-exercise period. The renal hemodynamic changes have returned to control values within 19 minutes, but the increased tubular reabsorption of sodium and chloride continued until the end of the experiment. Venous pressure before exercise was 3 mm. water; after exercise, 1 mm. water.

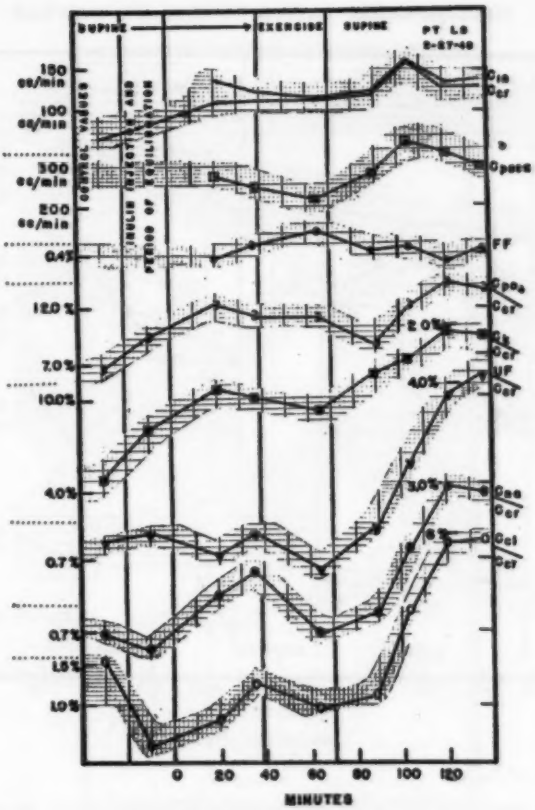


EXPERIMENT No. 6

Patient No. 1: L. S.

Considerable fluctuation occurred during this experiment in the values for filtration rate and renal plasma flow. The constancy of the filtration fraction would indicate that this is due again to imperfect urine collection. No marked alterations are seen in renal hemodynamics during exercise. Exercise (walking intermittently along ward corridor 400 yards in 27 minutes) produced some antidiuresis, while a depression in the ratios C_{Na}/C_{In}, C_{Cl}/C_{In} occurred, which in the case of sodium never reached its pre-exercise control level.

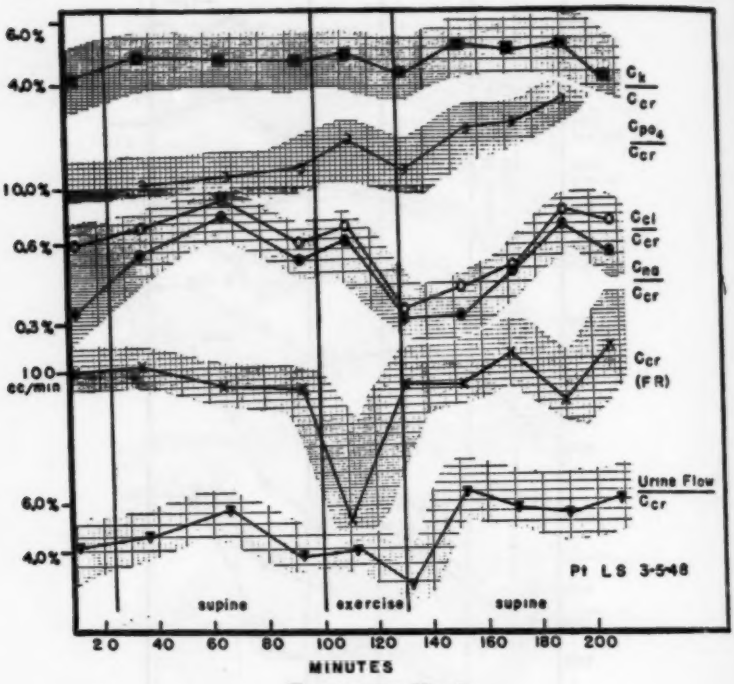
Venous pressure before exercise was 8 mm. water; after exercise, 2 mm. water.



EXPERIMENT NO. 7

Patient No. 1: L. S.

Experiment performed during period of supervision in the out-patient department. It will be seen that apart from the UF and Cl ratios, all electrolyte excretion ratios show a progressive rise from the initial control to the pre-exercise values. Exercise (walking intermittently along ward corridor 300 yards for 30 minutes) caused no change in filtration rate but a depression in renal plasma flow and the excretion of sodium and chloride. The mild antidiuretic effect of exercise was followed by increases in filtration rate, renal plasma flow and a four-fold change in urine volume. The effect of exercise in this experiment has been interpreted as a modification of the electrolyte excretion during the course of a spontaneous diuresis, thereby cutting a segment out of the progressively rising curves of all the clearance ratios. Venous pressure, before exercise was 115 mm. water, after exercise, 110 mm. water.

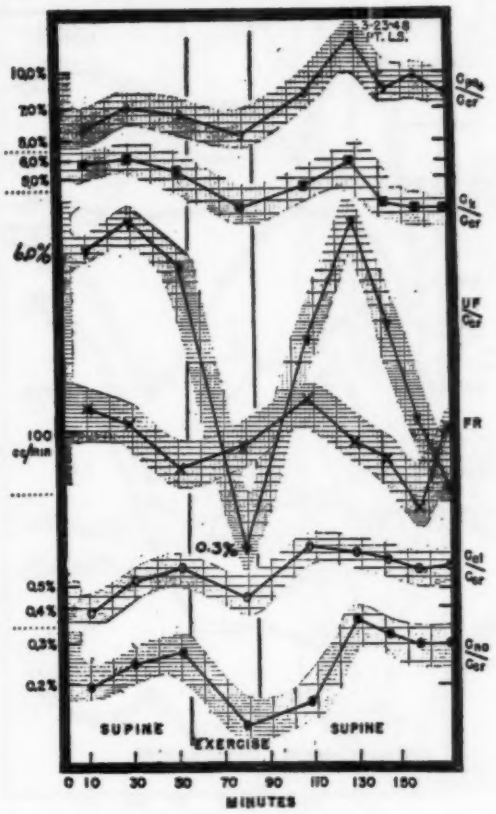


EXPERIMENT No. 8

Patient No. 1: L. S.

Second experiment performed during period of supervision in the out-patient department. Exercise comprised two eight-minute periods of quiet walking (total of 350 yards) along ward corridor separated by a ten minute standing rest period. Exercise caused an immediate fall in glomerular filtration, while the sodium and chloride ratios remained the same. Return of filtration rate to normal in the first post-exercise collection period was associated with depression of sodium and chloride excretion which returned to pre-exercise level 60 minutes after the end of walking. The clinical condition of the patient at the time of the experiment was not as good as in Experiment No. 7. Venous pressure before exercise was 185 mm. water; after exercise, 180 mm. water.

periments, exercise produced a constant and significant depression in the excretion of sodium and chloride, an effect which continued for an average of 40 minutes after the end of exercise. Filtration rate was depressed during exercise in Experiments No. 5, 8 and 11; showed

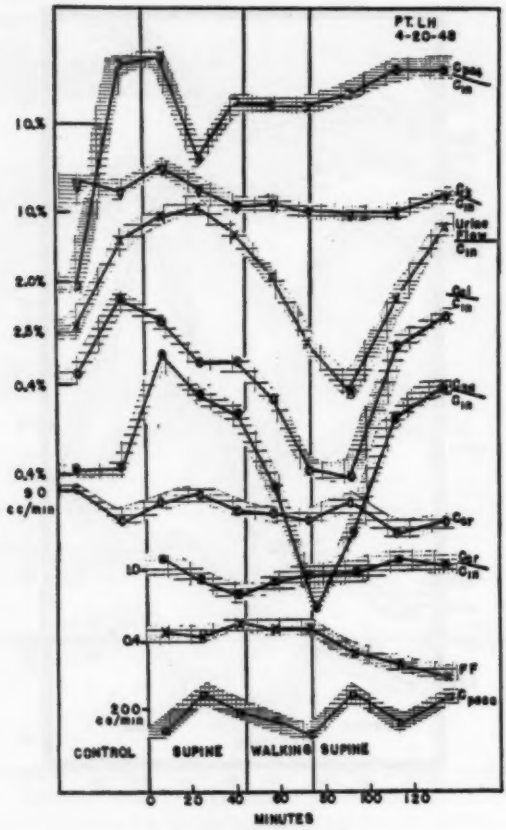


EXPERIMENT No. 9

Patient No. 1: L. S.

Third experiment performed during period of out-patient supervision. Exercise consisted of walking intermittently 300 yards in 30 minutes along ward corridor. Considerable variation is seen in the filtration rate throughout the entire experiment, due possibly, in this instance, to faulty urine collection. Exercise produced a marked antidiuresis, with depression of the C_{Na}/C_{Cr} and C_{Cl}/C_{Cr} ratios. Decreased excretion of sodium and chloride continued for a further 35 minutes after the end of exercise. Venous pressure before exercise was 118 mm. water; after exercise, 113 mm. water.

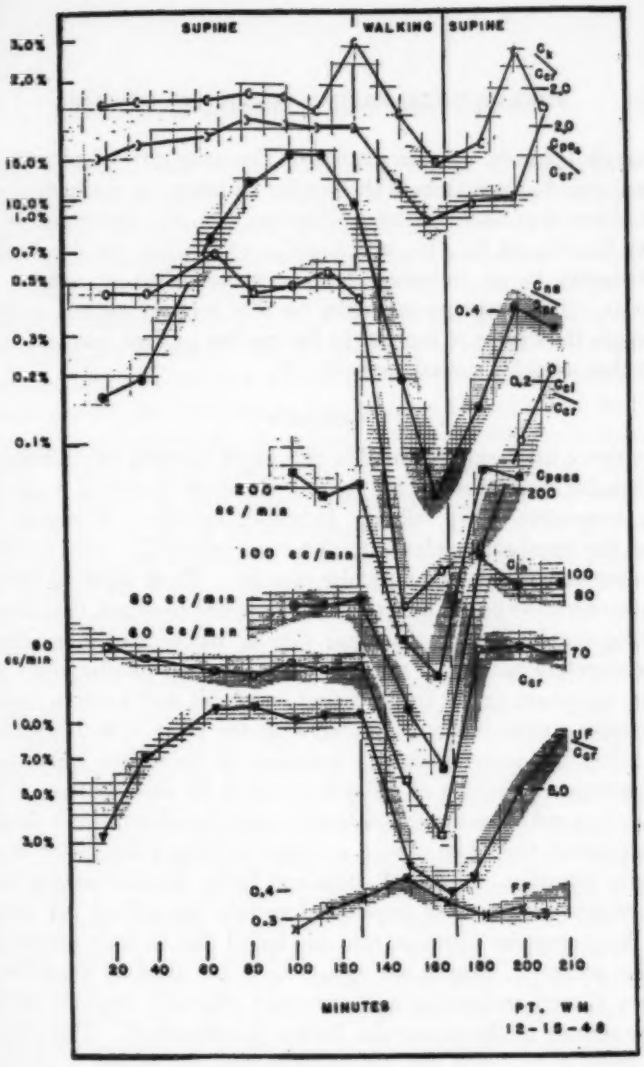
no significant change in Experiments No. 6, 7 and 10; while no deductions could be made in Experiments No. 4 and 9 due to variations in



EXPERIMENT NO. 10

Patient No. 2: L. H.

Control period represents 27 minute control urine collection plus one hour for injection and equilibration of inulin (10 gm.) and PACA (3 gm.). The three collection periods prior to exercise show constant filtration rate and fall in C_{Na}/C_{Cr} and C_{Cl}/C_{Cr} to initial control level, following the injection of the test substances. Exercise (walking 400 yards intermittently along ward corridor in 30 minutes) caused no alteration in filtration rate but produced a marked depression of sodium and chloride excretion. This effect is not abolished until the next two collection periods. Venous pressure before exercise was 87 mm. water; after exercise, 82 mm. water.



EXPERIMENT NO. 11

Patient No. 3: B. M.

Seven control periods were obtained prior to exercise; 10 gm of inulin and 3 gm. of PACA were injected at the end of the second urine collection period. Apart from the elevation of C_{Na}/C_{Cr} due to the injection of the test substances, filtration rate, renal plasma flow and all electrolyte clearance ratio are constant. During exercise (walking on a motor driven treadmill for 40 minutes at 1/2 mph.), filtration rate and renal plasma flow changed approximately six fold. An antidiuresis was associated with a fall of the ratio C_{Na}/C_{Cr} from 1.15% to 0.062% which returned to 0.4% 30 minutes after the end of exercise. Congestive cardiac failure in this patient was present with an average filtration rate of 75 cc./min. and a filtration fraction of 0.32.

the control periods. As the electrolyte clearance ratios depict changes caused only by variations in the tubular reabsorptive mechanism over and above that due to altered filtration rate, the depression of the ratios C_{Na}/C_{In} or C_{Na}/C_{Cr} and C_{Cl}/C_{In} or C_{Cl}/C_{Cr} are due in these experiments to an increased tubular reabsorption of sodium and chloride. This response is similar to that seen in normal subjects, although the degree of exercise in the cardiac patient was less severe than that used for normal controls (15).

DISCUSSION

Evidence has been presented in this paper showing the influence of the tubular mechanism in the control of sodium excretion in patients with congestive heart failure. Increased excretion of sodium followed the intravenous administration of aminophyllin, while retention was observed during mild muscular exercise. These opposing changes have occurred while the filtration rate remained constant, thus demonstrating the lability of the renal tubular mechanism in modifying the electrolyte pattern of the urine. This added tubular effect may be an important factor in the retention of salt and water in cases of congestive cardiac failure and differs from the views of Mokotoff, Ross and Leiter (12) who postulated a constancy in the sodium reabsorptive mechanism. Retention of sodium as stated by these authors is due largely to a reduction in the absolute amount of sodium filtered through the glomeruli, the cause of such a reduction being a depression of glomerular filtration. Mokotoff, Ross and Leiter demonstrated a linear relationship between the amount of sodium reabsorbed per minute and the glomerular filtration rate and found that in their control and cardiac group . . . "despite the variations in the absolute quantities of sodium filtered, reabsorbed and excreted a relatively constant fraction of the sodium in the glomerular filtrate is reabsorbed. This amount to a mean of 13.3 mm. per 100 cc. of glomerular filtrate". Analysis of our findings for the amount of sodium filtered, reabsorbed and excreted in the above experiments yields a figure of similar constancy. The constancy of this figure is of little significance because the amount of sodium excreted (mEq./min.) under any circumstance is of such a small magnitude when compared with the quantity reabsorbed that an apparent or relatively linear relationship between the sodium reabsorbed and the glomerular filtration rate is inevitable.

It may be questioned whether the interpretation of these authors represents the most revealing analysis of the data since it places emphasis solely on the variations in glomerular filtration rate with a fixed fraction of the filtrate reabsorbed in order to account for changing urinary sodium excretion. By expressing the urinary output of sodium as a clearance ratio ($C_{Na}/C_{In} \times 100$) changes in sodium excretion which are primarily due to variations in filtration rate have been eliminated. Under such a circumstance (if the changes in excretion of sodium are due only to variations in filtration rate with a fixed proportion reabsorbed) the ratio $C_{Na}/C_{In} \times 100$ would remain constant. Striking variations in this ratio have occurred in the experiments reported in this paper, demonstrating that the tubular cells play an active part in the economy of salt, either by an increased (see during muscular exercise) or a decreased percentage reabsorption (aminophyllin effect) of sodium in the glomerular filtrate.

A specific salt-retaining response to exercise appears to be a phenomenon common to both normal and cardiac subjects. Whether this response is elicited more readily and more intensely in cardiac patients requires further investigation. During various forms of mild exercise, Merrill and Cargill (19) found that six out of 10 cardiac subjects reacted with a reduction of filtration rate which approached or fell below a critical level of 70 cc./min. These authors state that below this level of filtration patients with a "normal tubular reabsorption of sodium retain salt and water when placed on an average salt intake." Mild exercise in the three cases of congestive failure reported in this paper produced varying results on the filtration rate, as the same individual (Patient L. S.) on different days showed either a reduction or no alteration in filtration rate. However, on all occasions a marked salt retaining response was elicited. As Merrill and Cargill point out, the limited amount of exercise used in the experimental method may be insufficient to tax the cardiac reserve of these patients and thus bring about detectable alterations either in renal plasma flow or filtration rate.

In 1944 Warren and Stead (10) reemphasized the concept of "forward failure" in the pathogenesis of peripheral cardiac edema; it being suggested that increased plasma volume as resulted from abnormal renal function. Confirmatory evidence was reported by Merrill (11) who showed a reduction in renal plasma flow proportionately greater

than the reduction in cardiac output in cases of congestive cardiac failure. It has been stated that salt and water retention occurs with filtration rates below 70 cc./min. (19).

Our results obtained from patient L. S. indicate that marked congestive failure can occur with a filtration rate within normal limits, although this patient had a reduction of renal blood flow sufficient to produce a filtration fraction of 0.54. Clinical improvement under digitalis, salt restriction and bed rest reduced this value to 0.23. However, with a filtration rate of 101 cc./min. and a plasma sodium clearance of 1.02 cc./min. when first determined at bed rest, this patient should have been able to maintain a normal sodium equilibrium. We infer, therefore, that the effect of daily activity with its salt-retaining response was responsible, in part, for her edematous condition. The hemodynamic change which seems to be correlated with salt retention is the high filtration fraction which in turn may influence tubular reabsorption. During Period No. 3 figures for sodium clearance show a progressive fall during a period of clinical deterioration. The filtration rate during this period did not show any depression from its previous values, while one determination of the filtration fraction showed a value of 0.49.

The present evidence suggests that if the mechanism of peripheral edema in congestive cardiac failure can be explained by the renal retention of sodium, the sequence of events can best be pictured in the following manner.

Firstly, in patients with a deficient cardiac output under stress, mild activity elicits more readily and intensely a salt-retaining response similar to that seen in normal subjects. Secondly, owing to a deficiency in cardiac output, less blood is diverted to the kidney. At this stage, through constriction of the efferent glomerular arterioles, glomerular filtration proceeds at a normal rate. Any retention of sodium and water at this stage would still be due to the increased tubular reabsorption of this electrolyte. Thirdly, in more advanced stages of cardiac decompensation and with greater stress, a reduction in glomerular filtration rate occurs, causing an added retention of sodium owing to a diminution in the total amount of sodium which is filtered through the glomeruli. That such a tubular effect does occur during the life of the ambulant cardiac patient is shown by the experi-

ments reported in this paper. We agree that it is probable that "altered reabsorptive faculties of the tubules precede the extravasation phenomenon at the peripheral capillary membrane" (20).

The mechanism of this salt-retaining response to muscular exercise has been considered elsewhere (15). It would appear not to be mediated by the posterior pituitary gland, although the antidiuretic effect of exercise has been shown by Verney (21) to be due to humoral influences of this gland. Smith and McKay (22) have demonstrated in normal subjects that the antidiuretic principle of the posterior pituitary causes an increase in the excretion of sodium and chloride with resulting negative balances of these elements. This effect is different from that which is observed during muscular exercise.

The known importance of the adrenal glands in the control of sodium excretion immediately suggests further mechanisms, but little is known of adreno-cortical activity in congestive cardiac failure at the present moment.

It has been emphasized that the excretory function of the kidney should be more closely allied to extracellular fluid volume (23), since the chemical anatomy of that compartment is controlled largely by renal mechanisms. Assuming constant renal function, the larger the extracellular fluid volume, the greater is the time required to clear a known amount of electrolyte from this space, because the electrolyte will be dispersed in low concentration through a large volume of fluid. Such delay in excretion of ingested sodium has been demonstrated on clinical grounds (8) and by the use of the radioactive element (9). Retention of sodium in an already edematous patient carries with it the inherent possibilities of progressive accumulation when viewed in the light of the theoretical relationships which exist between the various fluid compartments and the ability of the kidney to clear any electrolyte from the body. The fate of the edematous cardiac patient is closely allied with the ability of the kidney to maintain salt and water balance, to which end the renal tubular cells appear to play no insignificant role. In the words of Starling, "We see, then, that the production of dropsy in heart disease is by no means so simple as it first appears, but that it is due to a complicating series of interacting mechanisms all of which tend towards the death of the organism."

SUMMARY

1) Observations are reported on the effect of intravenous aminophyllin, mild muscular exercise and rest on the renal mechanism of electrolyte excretion in cases of congestive cardiac failure.

2) Electrolyte excretion has been expressed mainly by clearance ratios (the ratio of the clearance of electrolyte to the clearance of inulin or creatinine × 100) which records changes in the renal tubular reabsorption of electrolyte in terms of the filtered electrolyte not reabsorbed by the tubules.

3) Observations on one patient extended over a period of four months, including metabolic balance studies of sodium, chloride, potassium and nitrogen which were carried out for twenty-four days.

a) This case had a glomerular filtration rate within normal limits with a reduction in renal plasma flow and a high filtration fraction when first determined. With weight loss, diminution in peripheral venous pressure and sodium chloride loss under digitalis, bed rest and salt restriction, the filtration rate remained within normal limits, the renal plasma flow increased and the filtration fraction decreased to normal values.

b) The patient showed a positive nitrogen and potassium metabolic balance during the loss of sodium, chloride and weight. The amount of potassium retained was more than would ordinarily be expected due to retention of nitrogen. This suggests that disturbances in intracellular electrolyte and nitrogen structure may occur in congestive cardiac failure, reflecting an injury to the body cells and should be further investigated.

4) Mild exercise in three cases of congestive cardiac failure evoked a sodium chloride retaining response, characterized by a decrease in the fraction of filtered sodium not reabsorbed by the renal tubules (clearance ratio). Exercise was sometimes accompanied by decreases in glomerular filtration rate; but constantly resulted in a decrease in the sodium clearance ratio. The decrease in sodium excreted persisted after return of filtration rate to normal or to its pre-exercise level.

5) The action of aminophyllin on the kidney in cardiac decompensation is due to two mechanisms and is modified by the degree of decompensation.

(a) A direct effect on the circulation, causing augmentation of glomerular filtration rate and renal plasma flow presumably due to increased cardiac output.

(b) A specific renal tubular effect, diminishing reabsorption of filtered sodium.

6) Speculation is made concerning the role of the salt retaining response of exercise in the pathogenesis of peripheral edema in congestive cardiac failure.

Acknowledgements: The authors are indebted to Dr. E. K. Marshall, Professor of Pharmacology, Drs. E. Cowles Andrus and John Eager Howard, Associate Professors of Medicine and Mr. Harry Eisenberg, Chemist, The Johns Hopkins Hospital for advice and assistance given for theoretical and metabolic problems encountered in this study.

BIBLIOGRAPHY

1. STARLING, E. H., Physiological factors involved in the causation of dropsy. Lancet, **1**, 1267, 1896.
2. STARLING, E. H., Some points in the pathology of heart disease. Lancet, **1**, 569, 1897.
3. STARLING, E. H., Fluids of body. Herter Lecture (New York, 1908), W. T. Keener and Co., 1909.
4. STARLING, E. H., The Linacre lecture on the law of the heart. London, Longmans, Green, 1918.
5. HARRISON, T. R., Failure of the circulation. Williams & Wilkins Co., Baltimore, 1939.
6. MCMICHAEL, J., Advances in internal medicine. Vol. 11, Interscience Publishers Inc., New York, 1947.
7. FREEMONT-SMITH, F., The mechanism of water diuresis in man. Proc. Soc. Clin. Invest., **9**, 7, 1930.
8. FUTCHER, P. H. AND SCHROEDER, H. A., Studies on congestive heart failure. II. Impaired renal excretion of sodium chloride. Am. J. Med. Sciences, **52**, 204, 1942.
9. BURCH, G., REASER, P. AND CRONVICH, J., Rates of sodium turnover in normal subjects and in patients with congestive heart failure. J. Lab. Clin. Med., **32**, 1109, 1047.
10. WARREN, J. V. AND STEAD, E., Fluid dynamics in chronic congestive heart failure, interpretation of mechanism producing edema, increased plasma volume and elevated venous pressure in certain patients with prolonged congestive failure. Arch. Int. Med., **73**, 138, 1944.

11. MERRILL, A. J., Edema and decreased renal blood flow in patients with chronic congestive heart failure, evidence of "forward failure" as the primary cause. J. Clin. Invest., **25**, 389, 1946.
12. MOKOTOFF, R., ROSS, G. AND LEITER, L., Renal plasma flow and sodium reabsorption and excretion in congestive heart failure. J. Clin. Invest., **27**, 1, 1948.
13. NEWMAN, E. V., Therapeutic conference. The treatment of heart failure. Part II. The use of diuretics. Bull. Johns Hopkins Hosp., **81**, 430, 1948.
14. KATTUS, A., SINCLAIR-SMITH, B., GENEST, J. AND NEWMAN, E. V., The renal tubular reabsorption of salt with exercise in a patient with cardiac failure and normal controls. Abstrs. Am. Soc. for Clin. Invest., Atlantic City, May, 1948.
15. KATTUS, A., SINCLAIR-SMITH, B., GENEST, J. AND NEWMAN, E. V., The effect of exercise on renal circulation and electrolyte excretion in normal subjects. Bull. Johns Hopkins Hosp. (in press).
16. REIFENSTEIN, E. C., JR., ALBRIGHT, F. AND WELLS, S. L., The accumulation, interpretation and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus and nitrogen. J. Endocrinology, **5**, 367, 1945.
17. HARRISON, H. E., DARROW, D. C. AND YANNET, H., The total electrolyte content of animals and its probable relation to the distribution of body water. J. Biol. Chem., **113**, 515, 1936.
18. HOWARTH, S., MCMICHAEL, J. AND SHARPEY-SCHAFFER, E., The circulatory action of theophylline ethylene diamine. Clinical Science, **6**, 125, 1947.
19. MERRILL, A. J. AND CARGILL, W. H., The effect of exercise on renal plasma flow and filtration rate of normal and cardiac subjects. J. Clin. Invest., **27**, 272, 1948.
20. FARNSWORTH, E. B., Electrolyte partition in patients with edema of various origins. Am. J. Med., **4**, 338, 1948.
21. VERNEY, E. B., Absorption and excretion of water. The antidiuretic hormone. Lancet, **2**, 739, 1946.
22. SMITH, F. AND MACKAY, E. M., Influence of posterior pituitary extracts on sodium balance in normal subject and in patient with diabetes insipidus. Proc. Soc. Exper. Biol. & Med., **34**, 116, 1936.
23. NEWMAN, E. V., BORDLEY, J. AND WINTERNITZ, J., The interrelationships of glomerular filtration rate (mannitol clearance), extracellular fluid volume, surface area of the body and plasma concentration of mannitol. Bull. Johns Hopkins Hosp., **75**, 253, 1944.

MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Monday, January 10, 1949, at 8:15 P.M.

Hurd Hall, The Johns Hopkins Hospital

Dr. Follis: I would like to open this, the third meeting of the Johns Hopkins Medical Society for this year. The first speaker will be Dr. C. L. Conley.

AMINOPTERIN IN THE TREATMENT OF ACUTE LEUKEMIA

Since April, 1948, nine consecutive cases of acute leukemia have been treated with aminopterin (4-amino pteroylglutamic acid). Three of the patients were children and six were adults. Two cases were classified as acute lymphogenous leukemia, the remainder as acute myelogenous. Two patients showed no response to the drug, either clinically or hematologically, except for the development of stomatitis. The others showed a prompt fall in the white count, at times to extreme leukopenic levels. In no case did abnormal cells disappear from the blood, nor did the marrow alter from a leukemic pattern. One patient developed cells resembling megaloblasts in the marrow after 3 weeks of therapy. Several patients displayed transient reduction in the size of lymph nodes and spleen, but in only one patient was there a rise in hematocrit and platelets during therapy. No striking clinical remission was observed. Toxic manifestations were common and included stomatitis, diarrhea with bloody stools, and severe depression of the marrow. Individual patients showed marked variation in their tolerance to the drug. Six of the 9 patients are dead. Autopsies on 3 patients showed no pathologic changes which could definitely be attributed to the therapy.

Dr. Follis: Dr. Conley's paper is now open for discussion.

Dr. Schoenbach: I think it is indeed a privilege to hear someone of Dr. Conley's capabilities discuss this subject. There are a few matters I would like to have clarified. Of these cases studied, there is one which was not included. It was a case of acute leukemia. The child who was treated was acutely ill and there was marked bone change. The child showed a remarkable response and at the present time there is question whether he has leukemia at all. I would like to know why, several months later, that case was not included in a series of consecutive cases.

The term "antifolic acid compound" was used throughout. There is no evidence that its action, either versus neoplasms or leukemia, can be directly attributed to its antifolic acid activity. The compound has antifolic acid properties, but it also has been shown to inhibit the action of other essential metabolites as well as other steroid hormones.

I think we need far more critical study of these leukemic diseases. We should have more objective criteria for classification based more on function than on morphology. Perhaps the chemist can then tell us whether he can inhibit that metabolic function, or process. This may enable one to select the cases which will respond.

Aminopterin is a toxic compound. How much of the toxicity is due to its specific action and how much is non-specific one cannot evaluate until its pharmacology is known.

The third most important fact about toxicity is that we are looking for a differential effect upon neoplastic and normal cells. Animals are reproducible to a certain degree as to age, weight, sex, etc. One can inoculate them with a tumor whose biology is known and one can achieve remarkable histologic and developmental changes with doses of aminopterin far removed from the toxic effects.

I think the problem should really remain at that point for a long period of time, because if one could find what metabolic process has now been inhibited one would have a clue as to the necessary metabolic function in the neoplastic cell which apparently is not required by the normal cell. With that clue in mind, we would have some knowledge of what mode of approach to take in the development of future chemical compounds, which are far more active and far less toxic.

I think we should look at these patients who have received aminopterin as an extension of basic laboratory investigations. Certainly 4-aminofolic acid, and closely related compounds, are not yet chemotherapeutic agents for the treatment of leukemia.

Dr. Conley: Of two patients omitted, one was a case of Hodgkin's disease and we presented here only cases of acute leukemia, and the other was a seven-year-old boy who showed many symptoms of acute leukemia but never presented a leukemoid blood picture. Several attempts at biopsy failed to produce satisfacfactory material, so diagnosis was never established.

Dr. Schoenbach: Were the biopsies all made after treatment?

Dr. Conley: Yes.

Dr. Schoenbach: You performed an aspiration before treatment, which was typical of acute leukemia.

Dr. Follis: Is there any further discussion of Dr. Conley's paper?

If not, we will go on to the second paper on tonight's program by Drs. R. J. Bing, M. Hammond, J. Handelsman, S. Powers and F. Spencer. Dr. Bing will present the paper.

CORONARY BLOOD FLOW, CARDIAC OXYGEN CONSUMPTION AND CARDIAC EFFICIENCY IN MAN

The work reported is the outgrowth of the chance observation that the coronary sinus of man can be intubated through the right auricle. An attempt was consequently made to utilize this technique for the measurement of coronary blood flow in man. In order to accomplish this, it was decided to embark on a joint cooperative program with Dr. Eckenhoff and his associates from the Department of Pharmacology in Philadelphia and with Dr. Goodale of the Army Chemical Center at Edgewood, Maryland. These two groups of investigators had developed a procedure for the determination of coronary blood flow in the dog, combining the use of the nitrous oxide method with that of catheterization of the coronary sinus. The

nitrous oxide method, originally developed by Kety and Schmidt for the determination of cerebral blood flow, is applicable in principal to any organ from which a representative sample of venous blood can be obtained. The heart by virtue of its homogeneous structure and its venous drainage into the coronary sinus is particularly suitable. The coronary blood flow obtained with the nitrous oxide method represents the cc's of blood coursing through 100 grams of left ventricular muscle in one minute. By multiplying this volume by the difference in the oxygen content between arterial and coronary sinus blood, one obtains the oxygen contion of 100 grams of left ventricular tissue. The results obtained per 100 grams of cardiac tissue may be considered accurate. It should be emphasized, however, that values for left ventricular oxygen consumption and efficiency are approximations, since the weight of left ventricular muscle can be obtained only indirectly. It seems justified to estimate the left ventricular efficiency of the normal heart, since its weight can be obtained from available tables with a reasonable degree of accuracy. It is impossible, however, to calculate left ventricular efficiency in abnormal hearts, since the degree of hypertrophy cannot be determined. The calculation of left ventricular efficiency, using normal weights, will result in maximal values. Consequently, the finding of decreased efficiency is of significance,since the true efficiency must be even lower. This is the case in cardiac failure.

The studies were performed on normal individuals and on patients with anemia, coarctation of the aorta, essential hypertension, and heart failure due to mitral stenosis and insufficiency. In addition, two patients with aortic stenosis and insufficiency respectively were studied. Only 50 per cent of all attempted catheterizations of the coronary sinus were successful. This was probably due to anatomical variations in the structure of the right auricle; for instance, differences in the height of the Eustachian ridge.

The results showed that in normal man the coronary blood flow averaged 65 cc. per minute per 100 grams of cardiac tissue. The arteriovenous oxygen difference was 12 volumes per cent, and the oxygen consumption 7.8 cc. per 100 grams per minute. In anemia the coronary flow was increased, the arteriovenous oxygen difference and the oxygen consumption were reduced. In essential hypertension, mean values for coronary blood flow, the arteriovenous oxygen difference, and the oxygen consumption were normal (mean values 70 cc./min./100 grams, 10.6 vol. per cent, and 7.4 cc./100 grams/minute). In coarctation the coronary flow, the arteriovenous oxygen difference, and the oxygen consumption were markedly increased (mean values 83 cc./grams/min., 14 vols. per cent, and 11.6 cc./100 gms./min). In cardiac failure due to mitral stenosis and insufficiency the coronary flow was slightly reduced to a mean value of 60 cc./100 gms./minute, but the arteriovenous oxygen difference was increased to a mean value of 14.4 vols. per cent. The oxygen consumption per 100 grams of left ventricular tissue was slightly increased (8.6 cc./100 gms./min.). In the patients with aortic insufficiency the coronary blood flow and the cardiac oxygen consumption were increased. In contrast, in the patient with aortic stenosis the coronary blood flow and the cardiac oxygen consumption were within normal limits.

The per cent efficiency of normal left ventricular muscle (the ratio of left ventricular work and energy cost expressed in kilogram meters) averages 23 per cent. In failure due to mitral stenosis and insufficiency, the calculated maximal left ventricular efficiency is decreased. Consequently, true values must be even lower.

Dr. Follis: Dr. Bing and his collaborators' paper is now open for discussion.

Dr. Andrus: These results open up a very significant advance in the study of heart disease in man. As Dr. Bing has mentioned, the results obtained from studies made on the heart-lung preparation, are deficient in very important respects though they have undoubtedly contributed a great deal to the understanding of cardiac physiology. But the road toward discovery has been littered with disputes as to the significance of results which have been obtained with relation to cardiac failure.

The situation with regard to the understanding of heart failure, as attempts have been made to derive this from studies on the heart-lung preparation, is comparable to looking at a baseball game through a knothole in the fence. Conclusions depend a great deal upon which way the knothole points and which part of the field it is possible to view through it.

Dr. Bing has quite properly mentioned certain assumptions. I would like to have two of these clarified before I have entirely comfortable feelings about the results. The first has been the basis of some of the disputes regarding the mechanism of failure in the heart-lung preparation: Only about sixty per cent of the blood which enters the coronary arteries comes out through the coronary sinus. The remainder penetrates to the cavity of the ventricles through Thebesian channels which do not communicate with the coronary sinus. What is there about the nitrous oxide method that makes it possible to be certain that the catheter is getting a representative sample of the total coronary flow?

Another assumption mentioned: that the volume, or the weight, of cardiac muscle involved in the normal and in the failing heart is the same, of course, a very important one. If I understand the figures correctly, the assumption that the cardiac weight was increased in hearts with failure would only add to the significance of data in the same direction in which Dr. Bing has interpreted them.

I can't close without expressing my admiration for the energy and the enthusiasm and the patience which has been applied to this study. I hope that as he casts toward the coronary sinus Dr. Bing may get a higher percentage of strikes in the future.

Dr. Follis: Is there other discussion, or any other questions?

Dr. Schoenbach: There is one question I would like to ask which is essentially mathematical. There is an integral with limits, zero to "U", which is not explained. I would like to know what the zero to "U" was and how one derives that integral. I only saw the four points on the curve.

Dr. Follis: Is there any further discussion?

Dr. Rich: May I ask Dr. Bing a question which is in line with one of the questions that Dr. Andrus asked? I was most interested in the difference between the

coronary blood flow in heart failure in coarctation of the aorta and hypertension because so much depends there on this hundred grams. I wonder if Dr. Bing would tell us a little as to how he arrives at how many hundred grams there are.

Dr. Marksall: I would like to ask Dr. Bing, out of curiosity, how he determines the solubility of the nitrous oxide molecule.

Dr. Follis: Is there any further discussion of Dr. Bing's paper?

Dr. Bing: Dr. Andrus brings up the important question whether or not coronary sinus blood represents true mixed coronary vein blood. The question should be answered in the negative. The coronary sinus carries only blood which has perfused that part of the cardiac muscle which drains into the coronary sinus. Other portions of heart muscle may have a different oxygen extraction. Therefore, by means of the nitrous oxide method, we measure only the coronary blood flow and the oxygen consumption of that portion of the heart from which blood drains into the coronary sinus. This probably represents left ventricular muscle. The nitrous oxide method for the measurement of coronary blood flow would be invalidated if the coronary sinus contained mixed venous blood. However, such contamination can be suspected if the arterial and venous nitrous oxide concentrations fail to come to an equilibrium at the end of eight minutes.

Dr. Rich has put his finger on another weak spot when he asks how many hundred grams there are per total ventricular muscle. We calculate coronary blood flow and oxygen consumption per unit weight, since S, the partition coefficient, represents the ratio of nitrous oxide dissolved per gram of heart muscle to the amount dissolved in cc. of blood at a constant nitrous oxide tension and at 37 degrees. No assumptions are necessary in these calculations. However, in order to estimate how many hundred grams there are per left ventricular tissue, assumptions have to be made. There are several possible methods for the estimation of the weight of the left ventricle. One may estimate cardiac weight from x-ray data, using the anterior-posterior and lateral position. We found this unsatisfactory. We are now using the tables of Smith, who from several hundred autopsies related body weight to the minimal and maximal heart weight in normal individuals. It is obvious that such a table can only give approximate figures. The left ventricular weight is assumed to constitute 53 per cent of the total heart weight. As I said before, we omitted calculations of cardiac efficiency in abnormal hearts. But we have reasons to believe that the left ventricular efficiency in failure is low.

Concerning Dr. Marshall's question on the determination of the solubility of nitrous oxide in heart muscle: Eckenhoff and his associates used the procedure outlined by Kety and his co-workers for brain. They placed dog or human heart homogenized in a Waring blender in a tonometer and allowed it to come into equilibrium with nitrous oxide. The tissues were then analyzed for nitrous oxide in the Van Slyke apparatus.

To answer Dr. Schoenbach's question: When the nitrous oxide analyses are completed the values are plotted against time. The time of each sample is taken as the mid-point of the interval for which the sample was taken, except for the first

pair taken at a constant rate over the first minute (the integrated sample). Smooth curves are then drawn through the arterial and venous points. From these curves the values for arteriovenous nitrous oxide and the arterial venous nitrous oxide difference in blood are obtained at the end of each minute over the eight minute period.

Dr. Follis: The last paper, by Drs. W. E. Goodwin, R. D. Sloan, and W. W. Scott, is entitled "The Trueta Renal-Vascular 'Shunt'" and will be presented by Dr. Goodwin.

THE "TRUETA" RENAL VASCULAR "SHUNT"*

An Experimental Demonstration of Neurovascular Control of the Renal Circulation in the Rabbit, Cat, Dog, and Monkey

Trueta's concept of a renal vascular "shunt" mediated by the sympathetic nervous system was discussed, and some of the literature and early work pertaining to the subject was reviewed briefly.

The methods and results of experiments designed to study this concept in the kidneys of 40 rabbits, 40 dogs, 4 cats, and 4 monkeys were presented. The results of these experiments except in one case failed to show a reflex control of renal circulation with sciatic stimulation as described by Trueta. However, further experiments with direct splanchnic and renal nerve stimulation showed a marked neurovascular effect on the renal circulation with subsequent renal vasoconstriction varying from slight to almost complete ischaemia.

In some of the sections it is demonstrated that this ischaemia is first cortical with juxtamedullary glomeruli carrying the bulk of whatever blood is flowing through the kidneys. Some of the x-ray studies suggest the existence of cortical ischaemia with an increase of juxtamedullary and medullary concentration of the radiopaque dye in the stimulated kidneys as compared with the unstimulated control.

A brief moving picture in color made by Mr. William P. Didusch was shown. It recorded some of the animal experiments described in the paper.

This was considered to be a demonstration of a neurovascular control of the distribution of renal blood flow under the rather extreme and unphysiological conditions of these experiments.

Dr. Follis: Dr. Goodwin's and his collaborators' paper is now open for discussion.

Student: Dr. Goodwin, I believe in Truetta's monograph he mentioned that the blood from these juxtamedullary glomeruli went through the basi recti. Is that correct? (Dr. Goodwin answered in the affirmative.) In these experiments, I didn't notice any of the dye in the basi recti. I wonder if you have any explanation for that.

Dr. Goodwin: I don't have any explanation for that. We looked and looked

* To be published in *The Journal of Urology* Vol. 61, May 1949.

for that, and we never could understand whether it was because there was not enough dye getting in, or whether the time interval was wrong, or what was the difficulty. That was true of the india ink experiments. If done with trypan blue, or methylene blue, instead, which seem to mix better with the blood, you do get a bluing of the medulla with a pale cortex. However, that is apt to be a little confusing, because the controls sometimes look something like the experimental when those dyes were used, so we limited most of these to india ink, as we thought we could see it better under the microscope.

Dr. Grafflin: I should like to emphasize that while there is a very marked constriction of the glomeruli on the stimulated side in these experiments, the vasa recta do not stand out in a way at all comparable with those illustrated by Trueta and his group. In amplification of Dr. Goodwin's brief reference to Spanner's work, Spanner claimed to have demonstrated numerous arteriovenous anastomoses in the renal capsule, pelvis and cortex. Trueta *et al.* state that they could not confirm Spanner's observations, although it is not clear how deeply they went into the problem.

Dr. Follis: Is there any further discussion? If not, the meeting is adjourned.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Pathology. By W. A. D. ANDERSON AND COLLABORATORS, pp. 1426, $15.00. *C. V. Mosby Co., St. Louis,* 1948.

This volume edited by W. A. D. Anderson and 31 collaborators represents the most ambitious work on pathology attempted in this country to date. Because of the multiple authorship the material which is presented varies in quality. Some sections are excellent, while others are not at all impressive. However, each contains a great deal of information documented by an extensive bibliography, especially on pathologic anatomy; pathologic physiology is, in the main, inadequately treated. The book is arranged along traditional lines into General and Special Pathology. Such a scheme not only results in repetition but also fails to present a coherent story of disease. For instance, if one wishes to read about lymphogenous leukemia, he must turn to a number of sections, each written by a different author: *Blood, Bone Marrow, Spleen, Lymph Nodes,* etc. Perhaps the most objectionable characteristic of this book is its format. Much of the text is in extremely small print; when larger type is utilized it comes as a relief. However, the criteria employed in determining the point size for the subject in question are completely obscure. As a result of the typography, the pages in general are unattractive and make for difficult reading.

Because of its voluminous nature, for there are 1426 pages of text, much of which is in small print, and because of the arrangement of its subject matter, we would not care to recommend this work to the second year student of medicine who is meeting the subject for the first time. Nor would it seem particularly adapted to the needs of those specializing in clinical medicine since the necessary approach to an understanding of the biology of an entire disease is lacking. This volume, however, should be valuable to those few who are specializing in pathology and who, therefore, need a reference work in pathologic anatomy.

R.H.F., JR.

Liver Injury: Transactions of the Seventh Conference. F. W. HOFFBAUER, Editor. *New York, The Josiah Macy, Jr. Foundation,* 1948.

This small volume is a transcript of the latest of the stimulating conferences on liver injury held under the auspices of the Josiah Macy, Jr., Foundation. The papers presented were oncerned with the physiology of the liver and its alteration under experimental and diseased conditions. The topics discussed included the locus of formation of plasma proteins, the hepatic circulation and its surgical alteration, the regeneration of injured liver tissue, the mechanism and use of various tests of hepatic function, and the pathologic effects of plutonium on the liver.

The use of radioactive isotopes in the study of hepatic physiology is of particular interest. It is to be hoped that all the material presented will soon be available in more readily accessible periodicals.

O.D.R.

Introduction to Psychobiology and Psychiatry. ESTHER LORING RICHARDS, M.D. ScD. *The C. V. Mosby Company, St. Louis,* 1946. Second Edition. 419 pp. $3.75.

Dr. Richards' book is written for nursing and medical students with the aim of preparing each of these groups so that they become "sufficiently aware of major and minor sweeping emotional illnesses (psychoses and psychoneuroses) so that patients in the early stages of these illnesses can be competently treated or promptly referred elsewhere." Mere print cannot reflect the animation, humor and positiveness that Dr. Richards can achieve in her teaching, but there is enough of the flavor of her personality captured in this book to wake a good many active memories of her teaching in her former students.

The attitude toward the patient set forth in this book is indicated by the fact that no patient is referred to by his surname. For the psychiatrist who has come to feel that one of the primary requirements of good rapport is a sincere recognition of the individuality, sensitivity and self-respect of the patient who will, under treatment, become a collaborator in his own therapy, this attitude is disturbing. In child psychiatry, it is perhaps permissible to read, "Grace, a seven year old," but to find, "Miles was a man of fifty-eight," "Laura is a married teacher of thirty-eight," reflects an attitude toward the patient which many therapists feel is inimical to rapport. It is doubtful whether an attitude which places the patient in a dependent, rather lower class than his doctor or nurse is a good one to teach to students, however much it may lend itself to the kind of rapid treatment processes to which an extremely busy out-patient department can force psychiatrists. "Ben is a government employee of thirty-five making $4200 a year." A government employee with inadequate education but with sufficient force to reach such a salary deserves at least a "Mister" and his treatment for most psychiatrists would demand that his value as such be recognized. Many authors of nursing texts insist that nurses call all patients, particularly psychiatric ones, by their proper title and surname.

The section on psychobiology is designed to reflect Dr. Adolf Meyer's concepts. It follows the outline of his personality study form exactly. The patterns of biological development are presented very briefly—the psychobiology of infancy, childhood and adolescence are included from pages 39 to 43. In many areas concepts of constitutional determination of personality reactions are tacitly assumed where more psycho-dynamically minded students see possibilities, at least, of basic psychogenic factors. Dr. Richards has little patience with such philosophies of psychiatry; she is primarily concerned with the here and now of the patient's illness and the factors in it that can be dealt with directly and at once. In line with this, the treatment sections which are generally quite short, from less than a page

to no more than two, are concerned exclusively with very direct "advice and counsel."

The book contains some serious inaccuracies. In the section on the treatment of epilepsy this statement appears: "Specific drug therapy is confined pretty generally to luminal (phenobarbital) and sodium bromide." To be sure, Dilantin and Tridione are mentioned later on the page but the statement quoted was certainly not true in 1946. It was probably allowed to persist into the new edition as an oversight. A similar oversight is made in the statement that alcohol "acts first as an excitant and second as a depressant." The supporting material is all of the type generally used to sustain the view that alcohol is a depressant to the nervous system. In the appendix there are statements about the projective personality tests that are similarly in error.

To address the same book to students of medicine and student nurses is a most unusual procedure, though probably one that should more often be carried out. It accomplishes a much needed purpose in stressing that in medicine the physician and nurse are a team, each with his own special job to be sure, but each knowing how the other works and thinks.

The real value of Dr. Richards' book is the philosophy of service in medicine it depicts and which is so beautifully illustrated by the life of the author. On page 37 she says, "Doctors and nurses both are up against the most exacting of all occupations—one that taxes without let-up, head and body and heart." There is a real danger that willingness to give the full measure of devotion to the service of mankind may wane as the exact sciences entice the physician to greater objectivity. To physicians like Dr. Richards, what goes on in test tubes will always be only the handmaiden of a practice of medicine concerned with the health of living, feeling human beings.

P.V.L.

The Basis of Chemotherapy. By THOMAS S. AND ELIZABETH WORK. 435 pp. $6.50. *Interscience Publishers, Inc. 215 Fourth Ave. New York, N. Y.*

A completely rational chemotherapy of infectious disease is probably some distance in the future for as the authors remark: "The problem of reducing drug action to interference with any single enzyme is immense, and the study of intermediary metabolism is likely for the present to do more towards suggesting new types of drugs than towards solving the problem of the mode of action of known drugs." Thus it is, that most successful drugs have been at least partially happenstance, even when a studied attempt was made to find a new drug. The gradual evolution of atabrine from the known effect of methylene blue is such an instance. And yet the very intensive studies of antimalarials and their mode of action, which are nicely summarized in this book, do not explain how or why known antimalarials act.

The last decade or so has witnessed a tremendous application of all sorts of specialized disciplines to the general problem of chemotherapy. The dependence of the typhoid bacillus on tryptophane for growth, the genetics of a mold, neuro-

spora, growth factors for chicks, the formation of adaptive enzymes by bacteria, the toxicity of mercury, the absorption of viruses on cells, have all been fused into the modern "basis for chemotherapy."

This book is an excellent summary for all experimental workers interested in infectious diseases and their treatment. One of its chief merits is the combined consideration of results obtained in the chemotherapy of protozoan diseases with the more rapidly expanding bacterial chemotherapy. A bibliography of some 54 pages is not the least of its virtues.

F.B.B.

A History of the Heart and the Circulation. By FREDERICK A. WILLIUS AND THOMAS J. DRY. 456 pages, $8.00 *W. B. Saunders Company*, 1948.

As stated in the Preface, because "... the professional historians have been accorded several centuries to fulfill their obligations in this regard but have not as yet produced a comprehensive work" (on this subject matter) the authors feel that this book is justified and that "the interpretation of medical data, even those in remote eras, is in reality a function of the physician." The first quotation here is true beyond question, and there is much truth in the second statement.

The book is divided into three main sections, the first being a chronological presentation of brief biographical sketches of men who made contributions to the knowledge of the heart and circulation. In the second section there are special biographies of the twenty men chosen by the authors as having been the outstanding contributors to this field. The third section then divides the cardiovascular subject matter into eighteen parts and discusses the contributions to each chronologically. Except for the third section, each chapter is followed by a list of references to the outstanding works written by or on the men concerned.

The biographical sketches are written clearly and whereas the major emphasis is on each man's contribution to the cardiovascular field, enough is added about his personality and environment to make for fairly interesting reading. This is particularly true of the second section.

The book's chief virtue lies in its being a fairly comprehensive reference text. Because the authors are physicians and not historians, and because of the brevity of the book, a true picture of the men discussed and the relative value of their contributions has not always been given.

R.M.

Report of the Sanitary Commission of Massachusetts 1850. By LEMUEL SHATTUCK AND OTHERS. 321 pp., $4.50. *The Harvard University Press, Cambridge, Massachusetts.*

The clarity of vision which is necessary for a great work frequently comes from outside a profession. Such is the case in this magna charta of public health which was published originally almost a century ago, but which nevertheless is fresh and pertinent today. Lemuel Shattuck was primarily a bookseller and publisher, but was also interested in history and genealogy. His interest in the latter awakened

an interest in statistics and from that he was led to a sanitary survey of the city of Boston in 1845. In 1849, as a member of the state legislature he secured the appointment of three commissioners "to prepare and report to the next General Court a plan for a Sanitary Survey of the State." The other members besides himself were Nathaniel P. Banks, Jr. and Jehiel Abbott.

Each reader in going through this document will pick out very different items of interest—so wide is the survey. This reviewer was struck by the accounts of bilious remittant fevers occurring during the summer and fall in areas near mill ponds (pg. 74–76). These epidemics many competent people attributed directly to the existence of the dammed up streams by way of noxious effluvia. Descriptions of the epidemic and the situations make it likely that they were dealing with malaria.

A perusal of the mortality tables for Boston and for an interior country town shows clearly how much safer it was to live in the country than in a large city. Under the age of 5, the annual mortality *per cent* was 9.55 for males and 8.44 for females. It was 3.05 for both sexes in the country. Or to put it in the words of the report "In Boston, of those under five years of age, 9 out of every 100 died" in one year.

On page 98, the report points out that consumption "the dreadful disease is a constant visitor to all parts of our Commonwealth," but creates little alarm because it is so constantly present, whereas the occasional visit of cholera or some other epidemic disease creates alarm and, therefore, precautionary measures are taken. How true today that still is.

It is impossible to review each recommendation in the Report. A striking one is XVII "We recommend that in laying out new towns and villages, and in extending those already laid out, ample provision be made for a supply, in purity and abundance, of light, air and water; for drainage and sewerage, for paving, and for cleanliness". The economic waste and lack of sanitation involved in sending untreated sewage out into the rivers is carefully analyzed and it is suggested that methods be worked out to put this to use. Vaccination against smallpox is strongly supported.

The value of the book today lies not, however, merely in the things that are said, but in the cogent marshaling of facts to back up the arguments and in the clarity of the goal.

The Harvard University Press has performed a real service to all doctors and laymen interested in more than the individual patient. This report should, at the very least, be perused by all such.

F.B.B.

The Selected Writings of William Clowes. By F. N. L. POYNTER, pp. 179, 15s. *Harvey & Blythe Lmtd. London, W. I.* December 3, 1948.

William Clowes (1544–1604), an outstanding Elizabethan surgeon, served first in the Earl of Warwick's Army at LeHavre, then later became Surgeon to St. Bartholomew's Hospital. Finally, he became Surgeon to the Fleet which defeated

the Spanish Armada. A strong-willed man, he represented the best in any medical tradition for he believed in searching for knowledge anywhere—"That is to say, if I find either by reason or experience, anything that may be to the good of the patients, and better increase of my knowledge and skill in the art of surgery, be it either in Galen or Paracelsus, yea, Turk, Jew or any other infidel, I will not refuse it, but be thankful to God for the same."

He was not merely a barber surgeon, for he continually borrowed knowledge from his physician friends and used it whenever he thought it helpful. He wrote a book on the treatment of syphilis. He told his patients honestly when he could not cure them and whence came the source of their illness. "A few years past there repaired unto me a certain man infected with Lues Venerea. He earnestly desired me to tell him what his grief might be. I answered him secretly, that it came of a naughty, venomous matter. 'What do you mean by that'? said he, 'I pray you, speak plainly and openly, for here are none but these two gentlemen, my good friends'. It is a spice of the French disease' said I."

Like Paré he knew that when a patient recovered, that it was only that he had dressed him, and that God cured him. His forceful language—the first medical writings in the English tongue—is refreshing because it is not cluttered up with medical gibberish and involved rationale. He who would fain return to the use of his mother tongue in dealing with scientific subjects will find much delight here.

F.B.B.

BOOKS RECEIVED FOR REVIEW

A Handbook of Recorded Notations. Vocational Nursing. By ALICE L. PRICE. pp. 163, $2.00. *C. V. Mosby Co.*

An Introduction to Physics in Nursing. 179 pp. $3.25. *The C. V. Mosby Co., St. Louis.*

Introduction to Organic and Biological Chemistry, 2nd edition. By ARNOW AND REITZ. 795 pp. $5.75. *C. V. Mosby Co.*

Introduction to Psychobiology and Psychiatry, 2nd edition. By ESTHER LORING RICHARDS. 419 pp. $3.75. *C. V. Mosby Co.*

Measurements of the Public Health. By F. A. E. CREW. 243 pp. 10s. net. *Oliver and Boyd, Tweeddale Court, Edinburgh.*

Obstetric Analgesia and Anesthesia. By FRANKLIN F. SNYDER. 401 pp. $6.50. *W. B. Saunders Co., Philadelphia.*

Synopsis of Psychosomatic Diagnosis and Treatment. By FLANDERS DUNBAR. 501 pp. $6.50. *C. V. Mosby Co.*

Technic of Medication. By AUSTIN SMITH. pp. 255. $4.00. *J. B. Lippincott.*

Vocational Nursing for Home, School and Hospital. By ALICE L. PRICE. pp. 344, $4.00. *C. V. Mosby Co.*